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**08/125,407**      **22 September 1993 (22.09.93)**      **US**(71) Applicant: **THE BOARD OF TRUSTEES FOR THE LELAND STANFORD JUNIOR UNIVERSITY [US/US]; Suite 350, 900 Welch Road, Stanford, CA 94304-1850 (US).**(72) Inventors: **STEINMAN, Lawrence; 877 Lincoln Street, Palo Alto, CA 94301 (US). OKSENBERG, Jorge; 3625 Ramona Circle, Palo Alto, CA 94306 (US). BERNARD, Claud; 18 Trentwood Avenue, North Bald Win, VIC 3083 (AU). ZAMVIL, Scott; 1900 Belburn Drive, Belmont, CA 94002 (US). MITCHELL, Dennis, J.; 16880 Oak Glen, Morgan Hill, CA 95037 (US). KARIN, Nathan; Dept. of Neurology & Neurological Sciences, Beckman Center, Stanford, CA 94305 (US).**(74) Agents: **ROWLAND, Bertram, I et al.; Flehr, Hobbach, Test, Albritton & Herbert, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).**(81) Designated States: **AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).****Published***With international search report.  
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*(54) Title: **INTERACTION OF T-CELL RECEPTORS AND ANTIGEN IN AUTOIMMUNE DISEASE**

## (57) Abstract

Methods are provided for determining relations between autoimmune degenerative diseases and specific variable regions of T-cell receptors as associated with the host HLA or T-cells associated with combatting neoproliferative diseases. By identifying the particular T-cell receptors which cause or are the disease in mammals, various prophylactic and therapeutic techniques may be employed for inhibiting the attack of the T-cell receptors on the native protein or tissue to enhance the defense. In addition, individuals may be diagnosed as to their propensity for a particular autoimmune disease or the occurrence of such a disease.

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INTERACTION OF T-CELL RECEPTORS AND ANTIGEN IN AUTOIMMUNE  
DISEASE

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CROSS-REFERENCE TO RELATED APPLICATIONS

5        This application is a continuation-in-part of application Serial No. 08/066,325, filed May 21, 1993 which is a file wrapper continuation of application Serial No. 07/877,444, filed April 30, 1992, which is continuation-in-part of application Serial No. 07/517,245 filed May 1, 1990, and International Application Serial No. PCT/US91/02991  
10        filed May 1, 1991. This application is a continuation-in-part of Application Serial. No. 07/379,500 filed July 12, 1989, which is a continuation-in-part of Application Serial. No. 07/086,694, filed August 17, 1987, which disclosures are  
15        specifically incorporated herein by reference.

INTRODUCTION

Technical Field

The field of the subject invention is diagnosis and treatment of diseases, particularly autoimmune diseases.

20        Background

Autoimmune diseases are a result of a failure of the immune system to avoid recognition of self. The attack by the immune system of host cells can result in a large number of disorders, including such neural diseases as multiple

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sclerosis and myasthenia gravis diseases of the joints, such as rheumatoid arthritis, attacks on nucleic acids, as observed with systemic lupus erythematosus and such other diseases associated with various organs, as psoriasis, juvenile onset diabetes, Sjogren's disease, and thyroid disease. These diseases can have a variety of symptoms, which can vary from minor and irritating to life-threatening.

Despite the extensive research efforts that have been involved with elucidating the basis for these diseases, the diseases for the most part have been recalcitrant to an understanding of their etiology in the development of therapeutic modes. Many of the diseases are believed to be associated with lymphocytic involvement, which can result in attack and degradation of proteins, cytotoxicity, and the like.

In the case of cancer, tumor infiltrating lymphocytes (TIL) are believed to be part of the body's defense mechanism to destroy the tumor. Efforts have been made to expand T-cells found in tumor tissue and return the culture expanded cells to the host.

The complexity of the immune system has been a daunting barrier to an understanding of the autoimmune diseases and the immune response to neoproliferative diseases. In attempting to understand the mechanisms involved with the immunological response, there is substantial interest in understanding in what manner the system degenerates to attack self. By understanding the relationships between the components of the immune system, the manner in which the immune system distinguishes between self and non-self, and the components the immune system associated with a particular disease, ways may be developed to diagnose individuals who may be susceptible to autoimmune diseases and provide therapies to protect such susceptible individuals from autoimmune disease during its onset and

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during its progress or to treat individuals with specific T-cells.

### Relevant Literature

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterized by myelin destruction (McFarlin and McFarland, New Engl. J. Med. 307:1183- 1251 (1982)). At the site of demyelination, depletion of oligodendroglia cells and proliferation of astrocytes is usually observed. Raine and Traugott, Immunoregulatory Processes in Experimental Allergic Encephalomyelitis and Multiple Sclerosis, Elsevier, New York, 151-212 (1984); Prineas and Wright, Lab. Invest 38:409-421 (1978). There is an accumulation of morphologically identifiable macrophages, plasma cells and T lymphocytes, characteristic of an inflammatory response in the brain. Prineas, Handbook of Clinical Neurology, 3, Elsevier, New York, (1985) pp. 213-257. MHC Class II, positive antigen presenting cells and activated T-cells secreting various cytokines are present. Woodroffe et al., J. Neurol. Sci. 74, 135-152 (1986); Hafler and Weiner, Ann. Neurol. 22, 89-93 (1987); Hafler and Weiner, Immunol. Rev. 100, 307-332 (1987); Hoffman, J. Exp. Med. 170, 607-612 (1989). Several lines of evidence suggest that T lymphocytes migrate from the peripheral blood through the CNS compartment and participate directly in the promotion of brain lesions. Hoffman et al., J. Immunol. 136, 3239-3245 (1986); Traugott, J. Neuroimmunol. 4, 201-221 (1985). In studies of MS plaque tissue with monoclonal antibodies, it has been shown that the majority of T-cells have the helper inducer CD4 positive phenotype. Sobel et al., J. Exp. Med. 167, 1313-1322 (1988). Also, by restriction fragment length polymorphism analysis, T-cell receptor Va and V $\beta$  genes have been shown to contribute to the genetic control of susceptibility to this disease. Beall et al., J. Neuroimmunol. 21, 59-66 (1989); Seboun et al., Cell 57,

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1095-1100 (1989); Oksenberg et al., Proc. Natl. Acad. Sci. USA 86, 988-992 (1989) describe the use of TIL cells in the treatment of tumors (Barth et al., J. Immunol. 144, 1531 (1990)).

- 5 HLA-DR2Dw2 is associated with increased susceptibility to MS. Terasaki et al. Science 193:1245- 1247 (1976). Susceptibility to MS has been associated with certain MHC Class II genes. Oksenberg and Steinman, Current Opinion in Immunology 2:619-621 (1990). At the cellular level,  
10 oligoclonality of T-cells has been described in the cerebrospinal fluid (CSF) of MS patients. Lee et al., Ann. Neurol. 29:33-40 (1991). Oksenberg et al., Nature 345:344-346 (1990) describes the use of PCR to amplify TCR Va sequences from transcripts derived from MS brain lesions.  
15 Wucherpfennig et al. Science 248:1016-1019 (1990) and Ota et al., Nature 346:183 (1990) report studies of T-cell clones in man that recognize myelin basic protein.

#### SUMMARY OF THE INVENTION

- 20 The relationship of particular sequences of the Va and/or Vb subunits of the helper T-cell receptor is established by identifying invasive T-cells in tissue from autoimmune or neoplastic lesions. The particular variable regions may be identified from germline rearrangement, mRNA or the T-cell receptor subunit sequences. The oligoclonal  
25 regions of the T-cell receptor ("TCR") or the cells having such regions are then used for therapeutic applications for the treatment of the diseases.

- 30 With disease causing T-cells, peptides with the sequence of the T cell receptor or peptides with the sequence of the antigen bound by the T cell receptor may be used by themselves to block binding, for the formation of antibodies, or the preparation of cytotoxic molecules specific for the target T-cell. The amino acid sequence of the peptides may be modified for improved activity.

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Sequences based on motifs associated with the disease are provided for diagnosis and therapy.

In conjunction with the restricted repertoire of the TcR, the MHC phenotype is also relevant to susceptibility to particular autoimmune and neoplastic diseases. By screening for the presence of the susceptible phenotype, counseling and monitoring can be provided to minimize the occurrence and/or severity of the disease.

#### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for determining T-cell receptor variable regions related to autoimmune diseases. Also specific MHC profiles may be identified associated with specific autoimmune diseases, which will provide for monitoring of such patients to identify initiation of symptoms of such disease.

For autoimmune disease, by identifying specific T-cell receptor (TcR) variable regions associated with the disease, therapies are employed to inhibit the attack of the T-cells having such variable regions on the target cells or proteins. The therapies may involve ablation of T-cells carrying the particular variable regions, administration of agents associated with inhibition of the T-cell receptor to the target cell, or prevention of the degenerative effects of the binding of the T-cell to the target cell or protein. These agents include peptides with the sequence, or a modified sequence, of the T cell receptor or the antigen bound by the T cell receptor, which agents may be used to block binding, for the formation of antibodies, or the preparation of cytotoxic molecules specific for the target T-cell. For neoproliferative diseases, the T-cells having the appropriate T-cell receptor may be concentrated and expanded and returned to the host.

The T-cell receptor has two subunits involved in binding, either a and b, or g and d. The variable regions associated with the subunits have a similar organization to

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those of the immunoglobulins, the b and g subunits having a variable region which comprises exons associated with the V, D and J regions, while the a and d subunits comprise exons associated with the V and J regions. By rearrangement of germline DNA, the exons are joined to the constant or conserved region and by subsequent splicing of the messenger RNA, an open reading frame is achieved which encodes the subunit. Depending upon the particular genetic inheritance of the host, the spectrum of the variable region loci of an individual will be different from other individuals. In addition, not all variable region exons present may rearrange to form a functional T-cell receptor subunit.

For some autoimmune diseases, one may wish to distinguish between a lesion associated with a chronic condition or an acute condition. For example, for multiple sclerosis, the chronic condition is exemplified by the presence of large numbers of macrophages and a relatively low number of T-cells in comparison to acute phase which has lower levels of macrophages and higher levels of T-cells. The cells may be identified in accordance with conventional histocytochemistry techniques, using antibodies to surface markers, as appropriate.

By determining the loci which are rearranged to form functional variable regions, which variable regions are associated with autoimmune lesions, one can diagnose the nature of an autoimmune disease, establish the existence of a chronic episode, and treat the disease, prophylactically or therapeutically, by inhibiting the degenerative effect of the T-cells. By determining the loci which are rearranged to form functional variable regions effective against tumors, these cells may be used to combat the tumor.

T-cell receptors may be divided into two categories: the CD4 helper-inducer T-cell receptors, which T-cell receptors bind to Class II MHC; and the CD8 suppressor-cytotoxic T-cell receptors, which T-cell receptors bind to



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Class I MHC. For the most part, the T-cells associated with such diseases as multiple sclerosis are the CD4 positive phenotype, though CD8 can also play a role.

The autoimmune diseases of significant prevalence include multiple sclerosis, associated with destruction of myelin and glial cells, rheumatoid arthritis, associated with joint lesions, systemic lupus erythematosus (SLE), associated with the deposition of autoantibodies and immune complexes, psoriasis, pemphigus vulgaris, juvenile onset diabetes, associated with destruction of beta cells in islets of Langerhans, Sjogren's disease, thyroid disease, Hashimoto's thyroiditis, myasthenia gravis, as well as many others.

The variable (V) loci by themselves or in conjunction with the J loci of the T-cell receptors of T-cells found at the site of the lesion may be identified in a number of different ways. Particularly, the lesion or plaque is isolated and total RNA, from which cDNA may be prepared, or DNA is prepared according to standard procedures. To provide for more accurate results, the cDNA is amplified by any convenient technique, such as the polymerase chain reaction (PCR), cloning, or the like. In the case of the polymerase chain reaction, primers are employed which will identify the particular variable region which either has been expressed as identified by cDNA, or has been rearranged, so as to be associated with a J and C region.

Primers may be selected in accordance with the known sequences of conserved regions of the T-cell receptor subunits. It is found, that there will usually be 1 and no more than about 7, usually not more than about 5, generally only about 3 common, variable regions of the T-cell receptor subunits associated with the lesions. Therefore, with neural disorders, where the tissue is available from a deceased or from a biopsy having the particular disorder, one may relate the presence of T-cells in the lesions to the disease and, further, relate the particular V regions and J

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regions associated with the T-cells present in the lesion with the MHC antigen type of the deceased. In this manner, one may provide for identification of the variable regions of humans suffering from the disease with the MHC type and  
5 be able to treat the disease accordingly. With genomic DNA, one would establish the presence of the rearrangement in the DNA by having primers which relate to substantially conserved regions of the variable region and the joining of the constant region, either coding or non-coding regions.  
10 Alternatively, by cloning, one could sequence the DNA and establish the identity of the variable region. Isolation of mRNA from the tissue in question, reverse transcription to cDNA and then amplification and identification of the rearranged V-C product will also define the disease related  
15 marker. Similarly, with neoproliferative tissue, the tissue may be isolated and the T-cells effective for combating the neoproliferative cells identified.

Instead of the nucleic acid as the basis for the diagnosis, by having a battery of monoclonal antibodies,  
20 various techniques may be employed for identifying the binding of the antibody to the T-cells. Thus, flow cytometry, e.g. a FACS scan, may be employed, where the antibodies are labeled with a fluorescer and those T-cells to which the antibodies bind would then identify the  
25 particular variable region.

A 100% correlation is normally not to be expected, nor will it be necessarily achieved. It will usually be satisfactory that in at least 60%, preferably 70%, of the hosts positive for the disease, the shared variable region  
30 locus associated with the disease is present in a population of host T-cells, particularly in rearranged form. Similarly, in fewer than about 50%, preferably in fewer than about 30% of the hosts which do not present the symptoms of the disease, the rearranged variable region is absent.  
35 These percentages should be based upon a statistically significant number of hosts.

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T-cells with a shared variable region will express the variable region gene from a single V region subfamily. Gene segments which display greater than 75% nucleotide sequence similarity over the length of the V region gene are considered members of the same subfamily (Crew, et al (1981) Cell 25:59-66). The sequence similarity is calculated for the V region exon itself, and does not include sequences encoded by the D and J segments and N additions.

Depending upon the particular disease, various tissues may be employed for identifying the T-cells. For neural diseases such as multiple sclerosis, brain plaques or cerebrospinal fluid may be employed as a source of the T-cells. Similarly, for myasthenia gravis, muscle, thymus tissue or T-cells responsive to acetylcholine receptor may be employed. For rheumatoid arthritis, the synovium may be employed. For other diseases such as thyroiditis, or Grave's disease, thyroid tissue, or in systemic lupus erythematosus, kidney tissue may be employed as the source of T-cells.

Once the rearranged variable region(s) are established, one may then identify T-cell variable region allele(s) or T-cell receptors in a host, in association with the HLA or MHC restriction, as indicative of the propensity for the disease or the existence of the disease or the neoproliferative cell responsive T-cells. Where it appears that the disease is associated with a clonal or oligoclonal population of T-cells, the presence of one or more of the T-cell receptors having the rearranged variable regions associated with the disease will indicate the greater or lesser likelihood for the occurrence of the disease. A clonal population will have 100% of the T-cells expressing one  $V_\alpha$  or  $V_\beta$  region gene and one VDJ segment. An oligoclonal population will have from less than 100% of the T-cells expressing one  $V_\alpha$  or  $V_\beta$  region gene and one VDJ segment, to 50% of the T-cells expressing no more than 12  $V_\alpha$  or  $V_\beta$  region

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genes, with no more than six VDJ segments per expressed V region gene.

For diagnosis of autoimmune diseases, either the nucleic acid or antigen may be detected. For nucleic acid  
5 detection, DNA or RNA in cells may be isolated by any convenient means and by employing appropriate probes, in conjunction with techniques, such as Southern transfer, dot-blots, or the like, the presence of the rearranged V region may be detected. Depending upon the nature of the disease,  
10 there may be an opportunity for prophylactic intervention to reduce the potential for the disease occurring.

If one wished to determine the number of cells which are expressing the T-cell receptors associated with the disease, this can be achieved in a number of ways. The  
15 messenger RNA may be isolated from T-cells and probed with an appropriate probe for the V gene region. By employing Northern techniques, one can detect the presence of the messenger encoding the T-cell receptor and obtain a qualitative value for the amount of T-cell receptor being  
20 expressed containing the particular V region gene. Alternatively, one may prepare cDNA from the messenger and using the polymerase chain reaction, amplify the amount of messenger and determine the number of T-cells expressing the particular variable region in this manner.

25 More conveniently, one may use antibodies as described previously which are specific for the V region and/or the J region alleles or potentially the combination V-J for the a subunit. In this way, one may detect the V region and the J region, With the  $\beta$  subunit, intervention of the D region  
30 makes it unlikely to find a antibody which would be specific for the VDJ sequence, but the V and/or J region may be detected individually.

Antibodies may be prepared in accordance with conventional ways, particularly employing the monoclonal  
35 antibody techniques as described, for example in U.S. Patent

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Nos. 4,690,893; 4,713,325; 4,714,681; 4,716,111; and 4,720,459.

Any of a number of techniques may be employed for identifying the presence of a T-cell receptor binding to the particular monoclonal antibody or anti-serum. A wide variety of labels have been used for detection, such as particles, enzymes, chromophores, fluorophores, chemiluminescence, and the like. Any particular label or technique which is employed is not critical to this invention and any convenient technique may be employed. The techniques may be either competitive or non-competitive methodologies, including sandwich methodologies. The cells will usually be lysed to provide membrane-free proteins in accordance with conventional techniques. Cellular debris may be removed and the protein extracted and harvested. Alternatively, intact cells may be employed and detected by fluorescence activated cell sorting or the like.

For therapeutic purposes, there may be an interest in using human antibodies. Normally, one will not be permitted to immunize a human host with the T-cell receptor or fragment thereof to activate T-cells specific for the sequence of interest. However, there are alternatives, in that mice or other lower mammals may be immunized, and the genes encoding the variable regions of the antibodies specific for the T-cell region of interest isolated and manipulated by joining to an appropriate human constant region, and optionally, the complementary determining regions (CDR) used to replace the CDRs of a human antibody by genetic engineering. The resulting chimeric construct, comprising a lower mammal variable region or CDRs and a human constant region may then be transformed into a microorganism or mammalian host cell in culture, particularly a lymphocyte, and the hybrid antibodies expressed. Of particular interest would be IgG constant regions. See, for example, EPA 85.305604.2. Also recent techniques suggest random association of immunoglobulin

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genes from a human host for expression in a non-human cell host e.g. prokaryotic, and screening for affinity.

In some instances, it may be satisfactory to use mouse antibodies, where tolerance can be achieved or some degree of immune suppression may be involved. Immune suppression may be achieved with cyclosporine, irradiation, anti-leu3 (anti-CD4) (U.S. Patent No. 4,681,760), or the like.

The antibodies may be used in a variety of ways, for example, for inhibiting binding between the T-cell and the target cell, for killing of T-cells, or for isolating the T-cells. In the first situation, the entire antibody may be administered, or Fab fragments, or even only the Fv region. By removing all or a portion of the constant region, there may be a reduction in the immune response. For selectively killing the T-cells carrying the particular V region, one may use a variety of immunotoxins, which may include the antibody or specific binding fragment thereof, bonded to all or a portion of a plant toxin, such as ricin, abrin, etc., or diphtheria toxin. By employing an appropriate antibody isotype, e.g., IgM or IgG<sub>3</sub>, the complement cascade may be enlisted. Alternatively, a radioactive substituent may be used which provides for a lethal dosage upon binding of the antibody to the host cell. Another choice is to use an antibody or fragment thereof conjugated with a cytolytic agent for specific elimination of the undesired T-cells. Finally, the T-cell can be removed by extracorporeal means, such as plasmapheresis, where the plasma may be passed through or over antibodies bound to a support, with the undesired T-cells being selectively removed.

For therapeutic purposes, the antibody may be formulated with conventional pharmaceutically or pharmacologically acceptable vehicles for administration, conveniently by injection. Vehicles include deionized water, saline, phosphate-buffered saline, Ringer's solution, dextrose solution, Hank's solution, etc. Other additives may include additives to provide isotonicity,

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buffers, preservatives, and the like. The antibody or derivative thereof will usually be formulated in purified form at concentrations in the range of about 0.05 to 10  $\mu\text{g/ml}$ . The antibody may be administered parenterally, typically intravenously or intramuscularly, as a bolus, intermittently or in a continuous regimen.

Desirably, the dose should deplete or at least bind about 75% of the undesired T-cells, preferably at least about 90%. Typical doses for adult humans will be in a range of about 10 to 100 mg. Doses for children or other animal species may be extrapolated from the adult human dose based on relative body weight.

Instead of antibodies, oligopeptides may be employed, having the same or substantially the same sequence as the oligopeptide sequence identified as being diagnostic of the autoimmune disease. These sequences will be oligopeptides of at least 8, usually at least 10 more usually at least 12, and preferably at least 18 amino acids, and generally not more than about 60 amino acids, usually not more than about 50 amino acids, of the T-cell receptor subunit chain. While the entire subunit(s) may be employed, usually not more than about 50 number % of the amino acids will be employed, particularly excluding the conserved or constant region. All or at least a portion of the variable region, capable of binding to the target protein (the protein recognized by the T-cell receptor) and/or MHC antigen, will be present. The MHC antigen may be by itself or bound to a fragment of the target protein, which fragment will normally include the particular locus associated with the disease.

Of particular interest is the demonstration that the variable rearrangements of T-cells associated with specific sites of autoimmune disease have a restricted repertoire, so that a relatively small number of T-cell variable regions of both the a and b subunits will be observed. Furthermore, as will be discussed below, a limited repertoire of MHC type will be associated with the disease and as to these

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repertoires, there will generally be a restricted repertoire of T-cell receptor variable regions.

In particular, with MS patients it is found in the brain, particularly in the plaques associated with the disease, that T-cells may be isolated which have rearranged germline DNA to provide for expression of the T-cell receptor. This may be contrasted with brains of normal healthy individuals, where the cells found in the brain have unrearranged germline DNA.

By identifying a particular Class II haplotype or molecular phenotype, one can then identify particular Va and Vb variable regions associated with an autoimmune disease. Once the autoimmune associated T-cell receptor or variable regions are identified, one may then use the various therapies which are described in the subject application for prophylaxis or treatment.

As previously indicated, of particular interest are specific V regions and J regions of both the a and b chains of the T-cell receptor. For sequences of human and mouse V regions, see Concannon, et al., Proc. Natl. Acad. Sci. USA 83:6598-6602 (1986). Of the regions of interest of CD4 T-cell associated with multiple sclerosis, among Va families are 8-10, 12 and 16, particularly 10. Other regions of interest include 1, 5 and 7.

Of the J regions, of particular interest are Ja regions, more particularly the Ja region GGGTACCGAGATGACGAA-CCCACCTTTGGGACAGGCACTCAGCTAAAAGTGCAACTC.

Of the Vb regions, are the families 5, 6, 7 and 12 more particularly 5 and 6, and of the Vb 5 family, particularly 5.1 and 5.2.

In addition, certain amino acid sequence motifs are seen in the CDR3 region of the TCR. The sequences appear more frequently than would be expected and follow the sequence LCAS(S) (where the parentheses indicate the optional presence of the amino acid), particularly LCASS. The next amino acid will be one having a long chain,



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neutral, preferably hydrocarbon, such as L, I and V, or Q, particularly L. The next amino acid may be neutral or charged positively or negatively charged, may be short (2-4 carbon atoms) or long (5 to 6 carbon atoms), and may include  
5 G, A, P, L, I or V, as well as D, E, K, and R, particularly G, A, V, D and R, more particularly R and G, which are involved with sequences specific for the MBP sequence 87 to 106. The next amino acid will frequently be G, A or S, so that the motif will be L-X-(G, A or S), particularly (G).  
10 An alternative motif is P following LCAS(S), where the next amino acid is PT.

These peptides may be isolated free of flanking amino acids or may include up to a total of about 20 flanking amino acids. Alternatively, flanking regions may be  
15 provided which are not naturally occurring sequences. The peptides may be modified and used as described previously.

For the most part, the sequence will be derived from J<sub>h</sub> 1 and 2, more particularly 1.2, 1.6, 2.1, 2.3, 2.5, 2.6 and 2.7.

20 This same approach may be used to identify the rearrangement and expression of T-cell receptor subunits, both for restriction as to Class I and II MHC, to identify sequences associated with pathogenesis.

The presence of pathogenic T-cells may be detected with  
25 various probes, such as AGC CTA CGC; AGC TTG CGC; AGC CTG CGG; TTG CGC; and AGC CTA CGC AGC TTG CGC AGC CTG CGG TTG CGC (specific for LRGA). The probes may be as few as 6 nucleotides and as many as 30 nucleotides, usually being not more than about 31 nucleotides.

30 The peptides may serve as vaccines, to obtain an immune response, to ablate the pathogenic T-cells. Immune responses may be achieved in accordance with conventional ways. The peptides may be conjugated to an immunoassay, introduced into a viral vector so as to be fused to the  
35 envelope or capsid protein, fused to proteins using recombinant technology, and the like.

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The oligopeptide may be joined to other peptides, proteins, or polyalkyleneoxy compounds for a variety of reasons, such as to provide for enhanced stability, toleration, ease of preparation or purification, or the like. The subject peptides may be used to inhibit the binding of the T-cell receptor to the target peptide.

The peptide may be formulated in substantially the same manner as described for the antibodies. The amount of the active ingredient administered will vary widely depending on the particular composition, the particular host, the number and frequency of administrations, the manner of administration, etc. Usually there will be from about 0.01 to 10  $\mu\text{g/kg}$  of host, more usually from about 0.05 to 5  $\mu\text{g/kg}$  of host, where the concentration may range from about 10  $\mu\text{g/ml}$  to about 1  $\text{mg/ml}$ .

The manner of administration may vary widely, depending upon the formulation and nature of the active ingredient. Administration may be parenteral, intravascular, peritoneally, subcutaneous, oral, etc., may employ catheters, pumps, constant diffusion membranes, etc.

The oligopeptides may be prepared in a variety of ways, conveniently, in accordance with conventional synthetic procedures. Where larger sequences are involved, such as 30 amino acids or more, recombinant DNA techniques may be employed, where the gene may be synthesized in accordance with conventional ways, such as commercially available DNA synthesizers, expanded employing the polymerase chain reaction, and then inserted into an appropriate vector having the necessary transcriptional and translational initiation and termination regions. The resulting vector is then transformed into a host in which the expression vector is replicated and functional expression is obtained. The product may be secreted and harvested from the medium or when not secreted and retained cytoplasmically, the cells are harvested, lysed, and the desired protein isolated and purified in accordance with conventional ways.

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Instead of the oligopeptide, anti-idiotypic antibodies may be employed. By preparing a monoclonal antibody to the idiotype of the antibody to the subject oligopeptide, the anti-idiotypic may mimic the oligopeptide and serve to  
5 compete for the MHC with the T-cell receptor for the MHC antigen. The anti-idiotypic may provide greater stability on administration, as well as other advantages.

T-cells can be inhibited from reacting with MHC antigens which may result in pathogenesis by employing  
10 ribozymes specific for one or both subunits of the T-cell receptor. For the Class I TcR, the ribozyme would be directed against the  $\alpha$ -subunit, while for the Class II TcR, either the  $\alpha$ - and/or the  $\beta$ -subunit could be the target. The ribozyme would comprise a sequence having complementarity to  
15 the sequence encoding the CDR3. Unnatural nucleotides may be used to enhance stability, such as the presence of thio linkages or replacement of oxygen in the phosphate group with carbon groups or the like. Alternatively, antisense sequences could be used which were specific for the target  
20 subunits. Administration of the ribonucleotides would be in accordance with conventional means in relation to the transport of the ribonucleic acid across the blood-brain barrier.

The protective compositions may be used in vitro or in vivo by adding to groups of cells comprising lymphocytes and  
25 cells associated with the autoimmune disease or target protein. By adding the protective composition, usually a protein such as an antibody or peptide having the appropriate variable region sequence, one can prevent the  
30 destruction of the cells and/or target protein. Tissue destruction may result in the loss of cells, or in the loss of function in the cells that are present. Where cells are involved, the T-cells will be restricted by the major histocompatibility antigen of the target cells, with the  
35 target cells usually being syngeneic with the T-cells.

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Other oligopeptides are provided which are identified as inducing an autoimmune response to a self antigen, or a portion thereof, and are capable of binding to an MHC antigen of a host susceptible to the autoimmune disease.

- 5 The compositions may be employed to enhance protection, by serving to tolerize the host and prevent immune attack against the endogenous protein or cell producing the endogenous protein. For toleration, the subject peptides may be conjugated to syngeneic spleen cells, or be linked to  
10 an innocuous immunogen to which the host has been previously immunized, such as tetanus toxin, bovine serum albumin, etc. Adjuvants are normally avoided.

- Sequences which may be employed for toleration will be sequences from proteins endogenous to the host involved with  
15 autoimmune diseases, which include such proteins as the neurological proteins found in the peripheral nervous system (PNS) or the central nervous system (CNS) and the acetylcholine receptor (AChR). These proteins are designated as P<sub>0</sub> which is found in the PNS and CNS, P<sub>1</sub>, in  
20 myelin basic protein, the predominant CNS protein of myelin, P<sub>2</sub>, a predominant PNS myelin protein, PLP, a proteolipid protein, a PNS and CNS myelin constituent, and the acetylcholine receptor. P<sub>1</sub> is involved in post-immunization encephalomyelitis and may be involved in multiple sclerosis.  
25 P<sub>2</sub> is involved in post-immunization neuritis (Guillain-Barre syndrome) a major complication, for example, in the swine flu immunization program and the acetylcholine receptor is involved in myasthenia gravis and may play a role in post-immunization myositis. Other autoimmune diseases, such as  
30 rheumatoid arthritis, lupus erythematosus, myasthenia gravis, multiple sclerosis, post-immunization myositis, post-immunization neuritis, and juvenile diabetes are treated with the same methods.

- The particular protein of interest will be screened for  
35 the presence of a subject motif, and one or more sequences including the motif selected. Where the histocompatibility

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genotype (haplotype) of the intended recipient is known, one sequence may be preferred over another. However, where the haplotype is not known, or the composition may be administered to a number of different hosts, it will frequently be desirable to combine a number of the sequences as oligopeptides in the same composition. The oligopeptides may be present as the individual peptides, or may be joined together in a single sequence, with or without intervening bridges, where any bridges will be other than the naturally occurring intervening sequences of the immunogen. Desirably, any such sequence would have fewer than about 100 amino acids, more usually fewer than about 60 amino acids. If there are a plurality of motifs present in the immunogen, all or fewer than all of the sequences including the motifs may be employed in a single composition. Usually, there will be not more than ten different motif comprising oligopeptides, more usually not more than about six different oligopeptides in the composition.

There will usually be more than one partial sequence in the immunogen comprising the subject motif. The oligopeptide comprising the subject motif may be from any site of the immunogen sequence, that is N-terminal or C-terminal proximal or central, where the oligopeptide sequence will normally be substantially homologous with from 9-15 amino acids of the immunogen sequence, although longer sequences may also be employed. Usually, the difference in homology between the natural sequence and the oligopeptide which is employed will be not more than two lesions, more usually not more than 1 lesion, which may be insertions, deletions, or conservative or non-conservative substitutions. The composition may comprise one or more different oligopeptides, with the following sequence: charged amino acid, two hydrophobic amino acids, and at least one of the next two amino acids being a polar amino acid, where the charged or polar amino acid may be substituted by glycine, usually not more than one being

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substituted by glycine. The charged amino acids are aspartic acid, glutamic acid, lysine, arginine, and histidine (D, E, K, R, H). The hydrophobic amino acids are alanine, proline, valine, leucine, isoleucine, methionine, 5 phenylalanine, tryptophan, and tyrosine, that is both the aliphatic and aromatic neutral or substantially neutral amino acids having not more than one heteroatom, e.g., chalcogen, on the side chain (A, P, V, L, I, M, F, W, and Y). The polar amino acids will be the charged amino acids, 10 as well as serine, threonine, asparagine, and glutamine (S, T, N, and Q).

Usually, the motif sequence present in the oligopeptide will be at other than the C-terminus of the oligopeptide, desirably being at the N-terminus and not closer to the 15 C-terminus than the center of the sequence, where the second, third, or fourth amino acid of the motif (depending upon whether there are four or five amino acids in the motif) is the central amino acid. The N-terminal amino acid may be the same as the inducing peptide or may have an 20 internal amino acid of the inducing peptide as the N-terminal amino acid of the oligopeptide.

Alternatively, the oligopeptide sequence may be derived by binding assays, where an oligopeptide is selected which is capable of forming a ternary complex with the disease 25 inducing T-cell receptor and MHC molecule. The oligopeptides will usually have at least about nine amino acids and need not have more than about 30 amino acids, usually not having more than about 20 amino acids. The compositions are prepared in a variety of ways in accordance 30 with conventional synthetic techniques, particularly automated synthesizers. The subject oligopeptides may be joined covalently to other organic molecules, either proteinaceous or non-proteinaceous.

The oligopeptide sequence may be distinguished from the 35 natural sequence. In some cases sequence analogs will be prepared with stepwise substitution of the amino acids with

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alanine or valine, particularly alanine. Each of the peptides may then be tested for their binding affinity to a host Class II MHC associated with restriction of T-cells involved with the autoimmune disease. Once a substitution  
5 has been identified as reducing or enhancing MHC antigen binding affinity, the same site may be further substituted with other amino acids to determine whether further enhancement may be achieved. Amino acids associated with T-cell recognition may also be substituted to diminish T-cell  
10 stimulation. Thus, those amino acids of the oligopeptide associated with T-cell recognition may be modified to reduce T-cell stimulation in vivo, while not significantly affecting MHC antigen binding. In this way a strong blocking oligopeptide may be achieved, without inducing the  
15 autoimmune action of the T-cells. Generally the total number of amino acids substituted will not exceed 3, ranging from 1 to 3, usually 1 to 2. The reduction or enhancement in binding will usually be at least about 10-fold, more usually at least about 100 fold, and preferably at least  
20 about 1000-fold.

An alternative oligopeptide analog will have a functional group at the N-terminus, where the functional group would generally be from about 1 to 6, usually 1 to 3 carbon atoms, and may be alkyl or acyl, such as methyl,  
25 ethyl, propyl, isopropyl, hexyl, cyclohexyl, formyl, acetyl, propionyl or the like.

Depending upon the particular application, the subject compositions may be administered in a variety of ways, by themselves or in conjunction with various additives. Various  
30 carriers may be employed which are physiologically acceptable, such as water, alcohol, saline, phosphate buffered saline, sugar, mineral oil, etc. Other additives may also be included, such as stabilizers, detergents, flavoring agents, thickeners, etc. The amount of active  
35 ingredient administered will vary widely depending upon the particular composition, the particular host, the number and

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frequency of administrations, the manner of administration, etc. Usually, there will be from about 0.01 to 10  $\mu\text{g/kg}$  of host more usually from about 0.05 to 5  $\mu\text{g/kg}$  of host, where the concentration may range from 10  $\mu\text{g/ml}$  to 1  $\text{mg/ml}$ .

5 Transplantation or MHC antigens have polymorphic regions, where the individual alleles are associated with specific hosts. For the most part, the host will be diploid and heterozygous, so that each host will have two haplotypes, meaning that there will be two different copies  
10 of a particular transplantation antigen type from the same locus, unless the host is homozygous at that particular locus. Therefore, as to an individual host or a plurality of hosts, mixtures of oligopeptides will usually be employed. The subject oligopeptides may be administered  
15 concurrently or consecutively with the oligopeptides of the T-cell receptor.

For identifying T-cells associated with combating neoproliferative diseases, by identification of the variable region associated with such T-cells, tissue may be obtained  
20 by biopsy, surgical intervention or the like, the mRNA or DNA may be isolated from the tissue sample and in the case of mRNA, cDNA prepared in accordance with conventional ways. The DNA sample may then be assayed using a primer specific for the various Va or Vb regions to identify the predominant  
25 variable region associated with the disease. Once a number of patients have been screened, the likely variable region(s) associated with a particular tumor will have been identified. One may then use the affinity separations, e.g. panning, affinity chromatography, etc. for isolating the  
30 desired T-cells. The cells may then be expanded in culture using a conventional growth medium, with or without the addition of fetal calf serum, interleukins, e.g. IL-2, or the like. The cells may then be harvested after expansion by at least 100 and restored to the donor.



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Alternatively, one may have a stored supply of T- cells of the appropriate variable region and either matched or unmatched as to MHC, particularly Class I. These cells may then be administered by injection into the neoproliferative tissue site, or into the blood stream. From  $10^3$  to  $10^9$  cells may be administered to a human host in a physiologically acceptable medium, the amount varying with the number of cells available, the manner of administration, the frequency of administration, and the like.

10 In addition to identifying T-cell variable regions associated with specific autoimmune diseases such as multiple sclerosis, one may in addition identify specific molecular phenotypes associated with susceptibility to autoimmune disease. The phrase "molecular phenotype" is  
15 used instead of the designation "haplotype" since in the absence of segregation analysis in families, it cannot be certain whether these genes are all in a cis configuration on a single chromosome. A phenotype associated with multiple sclerosis is DRB1 1501, DQA1 0102 and DQB1 0602.  
20 This phenotype may be further broken down into 1a, associated with DPB1 0401 and 1b 0402. This particular phenotype is associated with  $V\beta$  family rearrangements, particularly  $V\beta$  5.1, 5.2 and 6.

By identifying MHC molecular phenotypes of individuals  
25 suffering an autoimmune disease, one can establish certain molecular phenotypes which provide for a susceptibility to the disease. In the case of multiple sclerosis one can look to see for rearrangement of T-cells and the prevalence of the various families and members of the families of the  $V\beta$  and  $V\alpha$  T-cell receptors. Once these are identified, one can  
30 use this information to ablate the T-cells associated with the disease. Thus, by isolating diseased tissue, e.g., plaques, and identifying T-cells having rearrangements, one can identify a family of  $V\beta$  and  $V\alpha$  regions which are  
35 associated with the disease, so that once the molecular phenotype has been identified as providing susceptibility to

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autoimmune disease one will also know which T-cells to ablate or target.

The upregulation of the MHC antigens on neural cells at lesions affords an opportunity to direct specifically various agents to the site of the lesion. Thus, one may use a variety of radionuclides, nmr agents, or other agents which provide a detectable signal for identifying the site of the lesion. The agent providing the signal may be joined to various carriers, such as antibodies for the MHC, or fragments of antibodies, e.g. Fab, Fv, etc., immunodominant sequences which are peptides of about 10, usually 12, amino acids or more, which have a high affinity for the MHC antigen. The presence of hematopoietic cells at the lesion, will further augment the presence of the agent at the lesion. The signal at the lesion should be greatly enhanced, as compared to other regions of the brain.

Besides diagnosis, one may use the selective presence of the MHC antigens for therapeutic purposes. Thus, one may direct various therapeutic agents, by conjugating the agent to the MHC antigen specific marker, by using the MHC antigen specific marker for directing therapeutic agent containing liposomes to the lesion site. Agents may include inhibitors of TNFa, down regulators of MHC antigen expression, e.g.  $\beta$ -interferon, TGF- $\beta$ , and  $\alpha$ -fetoprotein, peptides which block the MHC antigen-TcR interaction, inhibitors of generalized degradative pathways, such as reducing agents and superoxide dismutase for singlet oxygen, etc.

Since the disease results in some permeabilization of the blood-brain barrier, the opportunity to introduce drugs across the barrier is enhanced. One may still use injections at specific sites, permeabilizing agents, or employ naturally occurring transport mechanisms.

The various agents will be administered in accordance with their individual nature and in accordance with their purpose. Inert physiologically acceptable carriers may be employed, such as deionized water, saline, and the like.

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Concentrations and the use of other additives or components will be based on experience with like reagents and may be determined empirically.

The following examples are offered by way of  
5 illustration and not by of limitation.

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EXPERIMENTALI. TcR Va Expression in Brain Plaques of Multiple Sclerosis

Samples were taken from brain plaques of 3 patients with chronic progressive MS, and 3 controls (non MS). Total RNA and cDNA (from 5 µg RNA) were prepared according to standard procedures. cDNA was also prepared from 1 µg RNA isolated from a pool of peripheral blood lymphocytes from five different individuals, stimulated with 3 µg/ml of PHA. cDNAs were amplified by PCR for 40 cycles in the presence of 10 µCi of [<sup>32</sup>P]dATP (Amersham). Samples were analyzed by gel electrophoresis with ethidium bromide to identify the specific fragment band. After separation, bands were excised and radioactivity was determined. Results are expressed in median cpm. All TcR 5' primers amplify TCR sequences from germ line DNA using a specific 3' Va primer for each family. The following tables indicate the primers employed and the results.

Table 1. T-cell Receptor a Primers.

Primer	Clone	Sequence	Family Members
20 Va 1	HAP 10	5'-TTGCCCTGAGAGATGCCAGAG-3'	1.1, 1.2, 1.3
Va 2	HAP 26	5'-GTGTTCCCAGAGGGAGCCATTGCC-3'	2.1, 2.2
Va 3	HAP 05	5'-GGTGAACAGTCAACAGGGAGA-3'	3.1
Va 4	HAP 08	5'-ACAAGCATTACTGTACTCCTA-3'	4.1
Va 5	HAP 35	5'-GGCCCTGAACATTGAGGA-3'	5.1
25 Va 6	HAP 01	5'-GTCACCTTCTAGCCTGCTGA-3'	6.1
Va 7	HAP 21	5'-AGGAGCCATTGTCCAGATAAA-3'	7.1, 1.2
Va 8	HAP 41	5'-GGAGAGAATGTGGAGCAGCATC-3'	8.1, 1.2

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5	Va 9	HAP 36	5'-ATCTCAGTGCTTGTGATAATA-3'	9.1
	Va 10	HAP 58	5'-ACCCAGCTGGTGGAGCAGAGCCCT-3'	10.1
	Va 11	HAP 02	5'-AGAAAGCAAGGACCAAGTGTT-3'	11.1
	Va 12 (Ab13)	PGA 5	5'-CAGAAGGTAAGTCAAGCGCAGACT-3'	12.1
	Va 13	AB 11	5'-GCTTATGAGAACACTGCGT-3'	13.1
	Va 14	AB 21	5'-GCAGCTTCCCTTCCAGCAAT-3'	14.1
	Va 15	AC 24	5'-AGAACCTGACTGCCCAGGAA-3'	15.1
	Va 16	AE 212	5'-CATCTCCATGGACTCATATGA-3'	16.1
	Va 17	AF 211	5'-GACTATACTAACAGCATGT-3'	17.1
	Va 18	AC 9	5'-TGTCAGGCAATGACAAGG-3'	18.1
10	Ca (Ab51)	PGA 5	5'-AATAGGTCGACACACTTGTCACTGGA-3'	Ca

Table 2. T-Cell Receptor  $\alpha$  Expression in Brain Plaques of Multiple Sclerosis Patients.

Table 2. 1-Cell Receptor & Expression in Brain Fractions of Multiple Sclerosis Patients																				
	Vα 1	Vα 2	Vα 3	Vα 4	Vα 5	Vα 6	Vα 7	Vα 8	Vα 9	Vα 10	Vα 11	Vα 12	Vα 13	Vα 14	Vα 15	Vα 16	Vα 17	Vα 18	Vβ 8	Actin
Experiment No. 1																				
MS Br1	383	4640	760	520	240	850	826	1566	450	45860	5430	36380	3618	367	280	289	226	442	170	10445 0
MS Br2	140	824	523	310	830	415	660	23200	1750	29630	623	49125	456	220	317	12460	3572	338	280	79120
MS Br3	638	313	276	410	817	1520	210	15860	16310	21200	838	2050	302	225	462	3633	482	470	630	58358
C Br4	235	1100	135	115	286	7300	427	960	1036	317	560	726	485	278	466	630	545	830	900	65996
C Br5	580	875	180	490	110	846	160	324	780	120	344	138	762	755	876	860	715	570	860	66392
C Br6	137	290	133	530	836	640	910	110	140	350	670	1030	1095	2000	437	775	240	330	710	13933 7
Experiment No. 2																				
MS Br1	1650	3956	1450	790	547	545	1170	343	1856	32870	513	12978	866	868	3190	280	1048	1127	440	28592
MS Br2	967	340	1419	1575	3866	2837	1848	13373	2974	17337	1550	33020	1487	1072	3148	17968	1446	980	1338	22460
MS Br3	666	726	1198	790	1769	258	576	35220	18990	19138	948	2690	587	880	815	945	946	1570	630	22415
C Br4	1307	660	1740	1790	553	706	4540	4410	1333	584	919	763	860	206	590	713	2748	526	864	31282
C Br5	896	1670	2370	5000	2826	418	862	8175	2048	1307	1734	836	737	1040	2097	2925	1025	5276	4478	33018
C Br6	883	1727	716	865	610	1334	9514	1033	1256	1130	636	170	4636	1300	1930	1167	764	5915	370	29451
PBL (PHA)	9434	19464	8288	18434	18820	10483	12800	14886	13980	23040	11448	16968	16536	17750	30512	16544	21132	19732	ND	ND

The T-cell receptors present in the brain of MS patient 1 were amplified and subjected to gel electrophoresis, where control brain cDNA, MS parietal region brain cDNA, MS occipital region brain cDNA, PGAS, a full length TCR  $\alpha$  cDNA were compared. Actin sequences were successfully amplified from brain cDNA, but not from the PGAS 5 control using the following primers: (5'-ACG-A-A-G-A-C-G-A-C-C-A-C-C-G-C-C-CTG-3', 5'-CACG-T-T-G-T-G-G-T-G-A-C-G-CCGTC-3'). V $\alpha$  and C $\alpha$  transcripts were amplified from both MS brain cDNA and PGAS 5 templates, but not from the control MS brain cDNA with primers AB 13-14 (5'-CAG-A-AG-G-T-A-A-C-T-G-C-A-G-C-C-A-G-ACT-3', 5'-TTG-G-G-A-T-C-C-A-G-A-G-C-A-G-A-T-A-T-A-C-TGC-3'), and AB 41-42 (5'-CAG-A-A-C-C-C-T-G-A-C-C-C-T-G-C-C-G-T-G-TAC-3', 5'-GTG-T-C-C-A-C-A-G-T-T-T-A-G-G-T-T-C-G-T-A-T-C-TGT-3'. Rearranged TcR $\alpha$  sequences could be amplified from cDNA of the MS brain prepared from the occipital region using the V $\alpha$ 12.1 primer AB 13 and C $\alpha$  primer AB 42.

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The T-cell receptors present in the brain of MS patient 1 were amplified and subjected to gel electrophoresis, where control brain cDNA, MS parietal region brain cDNA, MS occipital region brain cDNA, PGA5, a full length TcR  $\alpha$  cDNA (Sim, *et al.*, *Nature* 312, 771-775 (1984)) were compared. Two  $\mu$ l of cDNA was combined in a 100  $\mu$ l reaction volume, with 1 unit of DNA Taq polymerase (Perkin Elmer-Cetus), 10  $\mu$ l 10x reaction buffer, 50  $\mu$ M each dNTPs, and 1  $\mu$ M of each primer. The PCR profile used was: denaturation 96°C for 60 sec., annealing 45°C for 60 sec. and extension 72°C for 120 sec., for a total of 35 cycles on a DNA Thermal Cycler (Perkin Elmer-Cetus). One tenth of each sample was independently run in a 4% Nusiev gel, and an appropriate size fraction was cut from the gel. The agarose piece was frozen and thawed 3 times, and 2  $\mu$ l of the supernatant were directly reamplified with the same primers for an additional 25 cycles. Actin sequences were successfully amplified from brain cDNA, but not from the PGA 5 control using the following primers: (5'-ACGAAGACGGACCACCGC-CCTG-3', 5'-CACGTTGTGGGTGACGCCGTC-3'). Va and Ca transcripts were amplified from both MS brain cDNA and PGA 5 templates, but not from the control MS brain cDNA with primers AB 13-14 (5'-CAGAAGGTAAGTGCAGCGCAGACT-3', 5'-TTGGGG-ATCCAGAGCACAGAAGTATACTGC-3'), which include the restriction sites PstI and BamHI and define a 286 bp fragment of the Val2.1 region gene and AB 41-42 (5'-CAGAACCCTGACCCTGCCGTGTAC-3', 5'-GTGTCCACAGTTTAGGTTCTGTATCTGT-3', which include a SalI site and define a 340 bp fragment of the Ca region transcript, respectively. Note that rearranged TcRa sequences could be amplified from cDNA of the MS brain prepared from the occipital region using the Val2.1 primer AB 13 and Ca primer AB 42.

Junctional region sequences were derived from the Val2.1-JC amplification from the occipital region of the MS brain. 100  $\mu$ l of the PCR reaction were phenol:chloroform extracted twice with 1/1 volume, chloroform extracted once

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with 1/1 volume, and dialyzed through a Centricon 30 (Amicon) with 2 ml of TE buffer for 30 min. at 5000 rpm. The sample was recovered and the DNA digested for 3 hr. with BamHI and PstI (or SalI). After digestion, the sample was  
5 phenol:chloroform extracted and then chloroform extracted once, passed through the same Centricon column as described above, and brought to a final volume of 20  $\mu$ l. 3  $\mu$ l of sample was put into a 10  $\mu$ l ligation with 200 ng of Bam HI/PstI or BamHI/SalI cut M13mpl8 and ligated overnight  
10 at 16°C with T4 DNA ligase (New England Biolabs). Transformation into E. coli JM101 was done according to standard procedures and positive plaques were selected by hybridization to <sup>32</sup>P-labelled TcR probes. 30 clones were sequenced by the dideoxy chain termination method using  
15 <sup>35</sup>S-dATP and Sequenase (U.S. Biochemicals).

The results in Table 2 show that in the amplification of the cDNA of one patient and the actin control, that actin could be amplified from the brain cDNAs but not from PGA5, a full length cDNA clone which contains the Val2.1 segment.  
20 Also, evident were lesser amounts of a smaller PCR product corresponding to the Val2.1 gene in the patient but not in the control sample. To ensure that the one Va family was amplified, genomic and brain Val2.1 PCR products were analyzed using restriction endonucleases and compared to the  
25 known restriction map. Only the expected fragments were observed, consistent with the notion that only the Val2.1 family was amplified. When colonies containing cloned Va PCR products were screened with a Val2.1 region probe, approximately 20% were positive. DNA from several of these  
30 colonies was sequenced and found to be identical to the published Val2.1 sequences (Sim, et al., Nature 312, 771-775 (1984)). Thus, the restriction fragment length polymorphisms (RFLP) recently associated with MS susceptibility must be in a sequence flanking to the TcR  
35 Va gene. (Oksenberg, et al., Proc. Natl. Acad. Sci. USA 86, 988-992 (1989)).



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The above results demonstrated that PCR could amplify the receptor transcripts from post-mortem brain samples, starting from 5 µg of total RNA without the necessity of in vitro expansion of T-cells. Similarly, Ca sequences were amplified from MS brain cDNAs, but not from the control sample. A subsequent amplification using primers complementary to the Va and the Ca TcR regions produced a major band when the control PGA5 and cDNA from the occipital region of the MS brain were used as a template, indicating the presence of rearranged TcR transcripts in the sample. The Va and Ca amplifications from the parietal region brain library most probably represent real transcripts from rearranged chromosomes, as has been found in other cDNA libraries from T-cell lines (Loh, et al., Science 243, 217-220 (1989)).

No PCR product was observed using primers corresponding to the Vb8 family, even though these primers are able to amplify the gene from buffy coat extracted genomic DNA. This TcR V region was recently reported to be associated with susceptibility to MS (Beall, et al., J. Neuroimmunol 21, 59-66 (1989)).

To provide further evidence that the DNA produced during the PCR amplification was an authentic amplified product of rearranged TcR genes, the PCR products were sequenced after double screening of colonies with Va and Ca probes. Only two different J regions were seen in the thirty sequences examined, both different from the PGA5 Ja sequence. Eleven sequences contain the Ja O family found in clone HAP41 (Yoshikai, et al., J. Exp. Med. 164, 90-103 (1986)). Fourteen sequences had a previously undescribed Ja sequence, GGGTACCGAGATGACGAACCCACCTTTGGGACAGGCACTCAGCTAAAAG-TGGAAGTC.

In order to completely analyze the TcR Va usage in MS brains, 18 different Va specific oligonucleotides for use as 5' PCR primers, based on published sequences for these gene families were prepared (Yoshikai, et al., supra (1986);

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Kline, et al., Proc. Natl. Acad. Sci. USA 84, 6884-6888 (1987)). Optimal conditions for amplification with each primer were ascertained with genomic DNA using a specific 3' Va primer for each TcR Va family and with reverse-transcribed RNA isolated from PHA stimulated peripheral blood lymphocytes. Using 5' Va primers and a common 3' Ca primer, the results from amplification of brain cDNA show that in each brain only a few TcR V gene families are preferentially expressed and rearranged. The Va 10 and 12 were detected in MS brains 1 and 2. MS brain 2 also expressed the Va 8 and the Va 16. In MS brain 3, the Va 8, 9 and 10 families were efficiently amplified. The Va 10 was thus common to all three samples.

In order to analyze the usage of Va genes, we analyzed cDNA reverse transcribed from mRNA isolated from uveal melanoma specimens. Eighteen different Va specific oligonucleotides representing the major human TCR Va families were used for the 5' primers and a Ca sequence was used for the 3'-primer (Table 1). Total RNA was extracted from each of eight uveal melanoma samples and was reverse transcribed. Total RNA from melanoma tissue was prepared in the presence of guanidinium thiocyanate in the method using RNazol™ (Cinna/Biotec, TX) (Choi, et al., Proc. Natl. Acad. Sci. USA 86, 8941 (1989)), and references cited therein). 2 µg of total RNA was used for the synthesis of single strand cDNA using reverse transcriptase. In a final volume of 20 µl 1xPCR buffer (50 mM KCl, 20 mM Tris-Cl, pH 8.4, 2.5 mM MgCl), 1 mM of NTP's, 20 units of RNasin, 100 pmoles of random hexamer (Pharmacia) and 200 units of BRL MoMuLV reverse transcriptase were incubated with RNA (2 µg) for 40 minutes at 42°C (Kamasaki, et al., Ibid., 85, 5698 (1988)). The reaction mixture was heated at 95°C for 5 minutes, then quickly chilled on ice. The DNA was then ready for PCR. The resulting cDNA was amplified using individual sets of Va- Ca primers with primers for melanotransferrin, a specific marker for melanoma. Each Va

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primer yielded a band of 300 to 400 bp on ethidium bromide-staining of the electrophoresed PCR product.

Analysis of Va expression in TIL from melanoma specimens is shown in Table 3.

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**Table 3. Usage of  
TCR Va Gene in Uveal Melanomas**

Case Number	Va Families									
	Va2		Va7							
1										
2		Va6	Va7					Val 3		
3			Va7					Val 3	Val 4	
4			Va7					Val 3	Val 4	
5			Va7							
6			Va7			Val 0				
7							Val 2			
8			Va7	Va8	Va9					Val 4

TCR Va families expressed in human uveal melanoma. A single stranded cDNA sample was amplified using Va-specific primer with a Ca primer at a final concentration of 1  $\mu$ M in each reaction. The amplification was performed with 2.5 units of Taq polymerase (ampli Taq™; Perkin Elmer) on a Perkin-Elmer DNA thermal cycler (Cetus). The PCR cycle profile was 95°C denaturation for 1 min. annealing of primers at 55°C for 1 min, extension primers at 72°C for 1 min for 35 cycles. PCR products were separated on 1% regular agarose/3% Nuseive™ agarose gels (FMC Corporation) and expression of Va families was considered positive when a rearranged band (300-400 bp) was visualized with ethidium-

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bromide staining. Experiments were repeated three times per sample. Results were identical with a different aliquot of each sample.

All TCR 5' primers amplify TCR sequences from germline DNA using a specific 3' Va primer for each family. We have detected a Va-Ca rearrangement of all TCR gene members in a variety of activated T-cells including single rearrangements of specific Va members in T-cell clones reactive to pertussis toxin, to Borrelia burgdorferi, and alloantigens as well as rearrangements of all Va members in pooled T- cells stimulated by PHA.

Among seven of eight cases only one to three Va genes (Va 7, 8, 9, 12 and 14) were detected. In seven of eight cases Va7 was expressed and rearranged. Melanotransferrin was amplified in all cases. Amplification artifacts due to contaminating DNA were excluded by performing controls in which no amplification was observed without cDNA samples or with genomic DNA. Identical results were obtained after a further experiment using different aliquots of each patient's tumor.

The amplified products obtained with the Va7 primer were further identified by hybridization with Va7 and Ca specific oligonucleotide probes. In all cases where the Va7 rearranged product was visualized on agarose gel electrophoresis with ethidium bromide staining, a positive hybridization was observed on dot blotting to the Va7 oligonucleotide probe (5'-CTG GAG CTC CTG TAG AAG GAG-3'). Amplified melanotransferrin did not hybridize with this probe at all. In addition, the Va7-Ca amplified product hybridized to a Ca oligonucleotide probe (5'-CAG AAC CCT GAC CCT GCC GTG TAC-3') but not with Va1 and Va4 specific oligonucleotide probes.

Additional characterization of the Va7 amplified products was obtained by restriction mapping with the endonucleases, DdeI, KpnI and HinfI. The restriction

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pattern was consistent with the known map of Va7. (Yoshikai, et al., J. Exp. Med., 114, 90 (1968)).

In this manner, the variable region(s) associated with each of the different neoproliferative tissues may be determined. A substantially homogeneous composition of T-cells may then be administered for treatment of the particular neoproliferative tissue. Instead of isolating mature T-cells, pre-T-cells may be isolated, activated with the tumor tissue or appropriate protein and the T-cells having the appropriate variable region(s) isolated and used for therapy. The T-cells may also be used for prophylaxis by administering T-cell compositions after chemotherapy, irradiation and/or surgical intervention.

TCR Va and Vb rearrangements were studied in 16 MS brains and in 10 control brains. TCRVa-Ja-Ca and Vb-Db-Jb-Cb rearrangements were confirmed with Southern blotting and hybridization of the PCR product obtained by amplification with 1 of 18 Va or 1 of 21 V $\beta$  specific oligonucleotide primers. The following table indicates the primers.

Table 4. T-Cell Receptor  $\alpha$  and  $\beta$  Primers.

Primer	Sequence	Primer	Sequence
V $\alpha$ 1	5'-TTGCCCTGAGAGATGCCAGAG-3'	VB 1	5'-GCACAACAGTTCCCTGACTTGAC-3'
V $\alpha$ 2	5'-GTGTTCCAGAGGGAGCCATTGCC-3'	VB 2	5'-TCATCAACCATGCAAGCCTGACCT-3'
V $\alpha$ 3	5'-GGTGAACAGTCAACAGGGAGA-3'	VB 3	5'-GTCTCTAGAGAGAAAGGAGCGC-3'
V $\alpha$ 4	5'-ACAAGCATTACTGTACTCCTA-3'	VB 4	5'-ACGATCCAGTGTCAAAGTCGT-3'
V $\alpha$ 5	5'-GGCCCTGAACATTTCAGGA-3'	VB 5.1	5'-ATACTTCAGTGTGAGACACAGAGA-3'
V $\alpha$ 6	5'-GTCACCTTCTAGCCTGCTGA-3'	VB 5.2	5'-TTCCCTAACTATAGCTCTGGCTG-3'
V $\alpha$ 7	5'-AGGAGCCATTGTCCAGATAAA-3'	VB 6	5'-AGGCCCTGAGGGATCCGTCCTC-3'
V $\alpha$ 8	5'-GGAGAGAAATGTGGAGCAGCATC-3'	VB 7	5'-CCTGAATGCCCAACAGCTCTC-3'
V $\alpha$ 9	5'-ATCTCAGTGCTTGTGATAATA-3'	VB 8	5'-ACTTTAAACAACAACGTTCCGA-3'
V $\alpha$ 10	5'-ACCCAGCTGCTGGAGCAGAGCCCT-3'	VB 9	5'-CTAAATCTCCAGACAAAGCTCAC-3'
V $\alpha$ 11	5'-AGAAAGCAAGGACCAAGTGT-3'	VB 10	5'-TCCAAAAAATCTCATCTGTACCT-3'
V $\alpha$ 12	5'-CAGAAAGGTAACTCAAGCGCAGACT-3'	VB 11	5'-TGTCTCTCAAAACCATGGCCATGAC-3'
V $\alpha$ 13	5'-GCTTATGAGAACACTGCCGT-3'	VB 12	5'-GATACTGACAAAGGAGAAGTCTCAGAT-3'
V $\alpha$ 14	5'-GCAGCTTCCCTTCCAGCAAT-3'	VB 13	5'-GGTGAGGGTACAACCTGCC-3'
V $\alpha$ 15	5'-AGAACTGACTGCCCCAGGAA-3'	VB 14	5'-ACCCAAGATACCTCATCACAG-3'
V $\alpha$ 16	5'-CATCTCCATGGACTCATATGA-3'	VB 15	5'-AGTGTCTCTCGACASGGCACAG-3'
V $\alpha$ 17	5'-GACTATACTAACAGCATGT-3'	VB 16	5'-CATGATAATCTTTATCGACGTGT-3'
V $\alpha$ 18	5'-TGTCAGGCAATGACAAGG-3'	VB 17	5'-AGCCCAATGAAAGGAACACAGTCAT-3'
		VB 18	5'-AGCCCAATGAAAGGACACAGTCAT-3'
		VB 19	5'-ACCCCGGAAAAGGACATACT-3'
		VB 20	5'-CTCTGAGGTGCCCCAGAA-3'
C $\alpha$	5'-AATAGGTCGACAGACTTGTCACTGGA-3'	CB	5'-TTCTGTATGGCTCAAACAG-3'

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Coded human brain samples were obtained from the Rocky Mountain Multiple Sclerosis Center in Englewood, Colorado and La Trobe University, Victoria, Australia. Samples included rapidly frozen and cryopreserved autopsy samples from different regions of 16 MS brains and 10 non-MS controls. Each sample was homogenized and the total RNA was extracted using the RNazol method (Cinna/Biotech, Friendswood, TX), (Chonczynski and Sacchi, Anal. Biochem. 162, 156 (1987)). Approximately 0.25  $\mu$ g of total RNA was reverse transcribed into a first cDNA strand in a 10  $\mu$ l reaction containing 1  $\mu$ l 10xPCR buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin (Perkin, Elmer, Norwalk, CT), 1  $\mu$ l of 10 mM dioxynucleotide triphosphates, 0.25 U random hexamers (Pharmacia, Piscataway, NJ) and 100 U of Superscript MuLV-reverse transcriptase (BRL, Gaithersburg, MD). The reaction mix was incubated at room temperature for 10 min, followed by incubations at 42°C for 45 minutes and 95°C for 5 minutes. The mix was then quick chilled on ice. cDNA was subjected to enzymatic amplification by the PCR method. 10  $\mu$ l cDNA was combined in a 50  $\mu$ l reaction mix with 4  $\mu$ l 10x PCR buffer, 1.25 U Taq polymerase, 0.5  $\mu$ M of Ca or Cb primer and 0.5  $\mu$ M of Va or Vb specific oligonucleotide primer (Table 4).

The PCR profile used was: Denaturation 95°C for 60 sec annealing 55°C for 60 sec and extension 72°C for 60 sec for 35 cycles in a DNA Thermal Cycler.

DNA Isolation and HLA Typing: High molecular weight DNA was extracted from tissue samples according to standard procedures. HLA-DRB1, DQA1, DQB1 and DPB1 typing was performed by PCR, dot blotting and hybridization with allele specific oligonucleotide probes (Helmuth, et al., Am. J. Hum. Genet. 47, 515 (1990); and Bugawan, et al., Immunogenetics 32, 231 (1990)).

Specificity was confirmed by identifying single rearrangements in antigen specific T-cell clones for B. bergdorfei, pertussis toxin and acetylcholine receptor. No



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amplification was detected in a colon carcinoma cell line. All samples were coded with the molecular biologist blinded to the origin of the specimen other than 3 MS brains and 3 controls.

- 5       A limited number of TCR Va gene arrangements were seen in 15 of 16 of the MS specimens.



TABLE 5 (Cont'd)

Sample	HLA-CLASS II					TCR V8															
	DRB1	DQA1	DQB1	DPB1	DR(Dw)	Plaque	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
MS																					
KL(C)	0601, 1501	0102, 0401/0601	04, 0602	0401, 0401	2(w2), w6	a	2	3	4	5	2	6	12								
						b	3	4	5	2	6	14									
						c	1	2	3	5	1	5	2	8							
LJ(C)	1101, 1501	0102, 0501	0301, 0602	0401, 0402	2(w2), w11	a	3	5	1	5	2	9	11	12							
						b	3					9	12								
						c															
						d	2	5	1				11								
						e	2	5	1				11	12							
HK(C)	1501, 1501	0102, 0102	0602, 0603	0401, 1401	2(w2), 2(w)	a	3	5	2	6	7	8									
ZD(C)	1101, 1501	0102, 0501	0301, 0602	0401, 0402	2(w2), 211	a	1	4	5	1	5	2	6	7	8	9	11				
TJ(C)	1501, 1601	0102, 0102	0502, 0602	0401, 0401	2(w2), 2(w21)	a	1	2	5	2	6	7	8	9							
MS2(A)	0101/2, 1501	0101, 0102	0501, 0602	0402, 0501	1, 2(w2)	a		5	1	7											
GL(A)	07, 1501	0102, 0201	0201, 0802	0401, 1101	2(w2), 7	a	3	5	2	6	7	8	9	12	13	14	15				
						b															
						c	3	4	5	2	6	7	8	9	10	12	13				
PM10(A)	07, 1501	0102, 0201	0201, 0602	0401, 0901	2(w2), 7	a	3	4	5	2	6	7	8	9	10	12	13				
					/1701	b	4	5	2	8	9										
TF(C)	0404, 1601	0102, 0301	0302, 0602	0201, 1101	2(w-), 4(w14)	a		5	2	6	7	8	14	15	17						

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TABLE 5 (Cont'd)

5	HLA-CLASS II										Plaque	TCR Va																	
	DRB1	DQA1	DOB1	DPB1	DR(DW)	1	2	3	4	5		6	7	8	9	10	11	12	13	14	15	16	17	18					
10	ZJ(C)	0301,0801 04/06, 04/05/06	0201,04	0401,0601 /1601	3,w6	a	1			5														16 17					
	RH(C)	0101/02, 0101,0101 1001	0501,0601	0201,0402	1,210	a	1			5														12					
15	HS1(A)	0301,1303 0401,0401	0201,0301	0101,0201	3,w13	a									10									12					
	HS4(A)	0301,1302 0102,0501	0201,0604	0201,0201	3,w13	a									8 9 10														
20	HY(A)	1303,1401 0101,0501	0301,0603	0201,0402	w13,w14	a						6					11							16					
						b				5							11	12						16					
25	K1(A)	0404,1301 0103,0301	0302,0803	0402,0601	4(w14),w13	a																		16					
	MS5(A)	0101/ 1401	0101,0101	0501,0603	0301,0401	1,w14	a																						
30	CONTROLS																												
	PH1125(A)	0402,1401 0101,0301	0302,0603	0401,0401	4(w10),w14																								
35	PH602(A)	0301,1302 0102,0501	0201,0804	0401,0601	3,w13																								
	PH1367(A)	ND																											
	C1(A)	ND																											
	C2(A)	ND																											

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TABLE 5 (Cont'd)

HLA-CLASS II															TCR VB												
Sample	DRB1	DQA1	DQB1	DPB1	DR(DW)	Plaque	1	2	3	4	5.1	5.2	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
5																											
10	ZJ(C)	0301,0801 04/06, 04/05/06	0201,04	0401,0601 /1601	0401,0601 3,w6	a	1	2	4						8	9	10	12									17
	RH(C)	0101/02, 1001	0101,0101	0501,0601	0201,0402 1,210	a								7													
15	MS1(A)	0301,1303	0401,0401	0201,0301	0101,0201 3,w13	a					ND																
	MS4(A)	0301,1302	0102,0501	0201,0604	0201,0201 3,w13	a					5.1			7	8												
20	HY(A)	1303,1401	0101,0501	0301,0603	0201,0402 w13,w14	a													12							18	
						b													12							18	
						c													12								
25	K1(A)	0404,1301	0103,0301	0302,0803	0402,0601 4(w14),w13	a																					
	MS5(A)	0101/ 1401	0101,0101	0501,0603	0301,0401 1,w14	a									6	8											
						b																					
30	CONTROLS																										
	PH1125(A)	0402,1401	0101,0301	0302,0603	0401,0401 4(w10),w14																						
	PH602(A)	0301,1302	0102,0501	0201,0804	0401,0601 3,w13																						
	PH1367(A)	ND																									
35	C1(A)	ND																									
	C2(A)	ND																									

TABLE 5 (Cont'd)

Sample	HLA-CLASS II					Plaque	TCR Vα																		
	DRB1	DOA1	DQB1	DPB1	DR(Dw)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
5																									
10	C3(A)	ND																							
	LV(C)	0404,0405	0301,0301	0302,04	0301,0401	4(w14),4(w15)																			
	HE(C)	0406,1301	0103,0301	0301,0603	0201,0401	4,w13																			
15	OR(C)	1402,1501	0102,0103	0502,0603	0301,0401	2(w-),w14																			
	HO(C)	0301,0401	0301,0501	0201,0301	0301,1101	3,4(w4)																			

TABLE 5 (Cont'd)

Sample	HLA-CLASS II					TCR V8															
	DRB1	DQA1	DRB1	DPB1	DR(Dw)	Plaque	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
5																					
10	C3(A)	ND																			
	LV(C)	0404, 0405	0301, 0301	0302, 04	0301, 0401	4(w14), 4(w15)															
	ME(C)	0406, 1301	0103, 0301	0301, 0603	0201, 0401	4, w13															
15	OR(C)	1402, 1501	0102, 0103	0502, 0603	0301, 0401	2(w-), w14															
	MO(C)	0301, 0401	0301, 0501	0201, 0301	0301, 1101	3, 4(w4)															
20																					

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In one specimen, no Va genes of the 18 families surveyed were rearranged, although this patient had two V $\beta$  rearrangements. The number of TCR Va genes transcribed ranged from 0 to 9 per brain, with a mean of  $4.4 \pm 2.8$  ( $\pm 1$  SD). TCR V $\beta$  rearrangements were more diverse, with a range of 2 to 13 per brain, with a mean of  $7.0 \pm 3.4$  ( $\pm 1$  SD). TCR Va or Vb transcripts were not detected in any of the 10 brains of individuals who died of non-neurologic diseases.

10 All of the 16 MS patients were typed for the Class II HLA loci HLA-DRB1, DQA1, DQB1 and DPB1 using PCR and sequence-specific oligonucleotide probe hybridization. Eight of 16 patients were DRB1\*1501, DQA1\*0102, DQB1\*0602 and either DPB1\*0401 or 0402. This molecular HLA-DR:DQ  
15 haplotype, which corresponds to the cellular type HLA-DR2Dw2, is associated with increased susceptibility to MS in certain caucasoid populations. Patients who were of the above indicated molecular phenotype showed an increased frequency of certain Va and Vb rearrangements.



Table 6. TCR V $\beta$  Gene Usage Correlated with HLA-DR2 Molecular Phenotypes.

TCR V $\beta$ Rearrangement	Phenotype 1*				Phenotype 1a $\S$				Phenotype+			
	(+) MS	(-) MS	Control(-)	Control(+)	(+) MS	(-) MS	Control (-)	Control (+)	(+) MS	(-) MS	Control (-)	Control (+)
V $\beta$ 5.1	4/8	3/7	0/7	3/7	3/7	2/8	0/7	0/7	3/38.h	2/128	0/7h	0/7
V $\beta$ 5.2	7/8 <sup>a,b</sup>	2/7 <sup>a</sup>	0/7 <sup>b</sup>	7/7 <sup>c,d</sup>	7/7 <sup>c,d</sup>	2/8 <sup>c</sup>	0/7 <sup>d</sup>	0/7 <sup>d</sup>	2/3	7/12	0/7	0/7
V $\beta$ 6	6/8	4/7	1/7	6/7 <sup>e,f</sup>	6/7 <sup>e,f</sup>	3/8 <sup>e</sup>	1/7 <sup>f</sup>	1/7 <sup>f</sup>	1/3	9/12	0/7	0/7

\*Phenotype 1 = HLA-DR2Dw2/DRB1\*1501/DQA1\*0102

 $\S$ Phenotype 1a = HLA-DR2DW2/DRB1\*1501/DQA1\*0102/DQB1\*0602/DPB1\*0401

+Phenotype 1b = HLA-DR2DW2/DRB1\*1501/DQA1\*0102/DQB1\*0602/DPB1\*0402

a  $\chi^2=5.4$ ,  $P<.025$ b $p=.002$  Fisher's Exact Testc $p=.011$  Fisher's Exact Testd $p=.0006$  Fisher's Exact Teste  $\chi^2=3.6$ ,  $p<.07$ f $p=.004$  Fisher's Exact Testg $p=.009$  Fisher's Exact Testh $p=.011$  Fisher's Exact Test

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- As evidenced by the above table, of 8 patients with the indicated phenotype, 7 had rearrangements of Vb 5.2 and all 8 rearranged either Vb 5.1 or 5.2 or both. Vb 6 was transcribed in 6 of 8 MS brains with the above phenotype compared to 4 of 8 MS brains of patients who were not of the indicated phenotype and 6 control brains which were not of the indicated phenotype. Other frequent rearrangements seen in patients with the indicated phenotype were Vb 7, 6/8, Vb 12, 4/8, Va 16, 6/8, Va 5, Va 7, Va 12 and Va 1 in 4/8.
- 10 Cloning and Sequencing of PCR-Amplified cDNA: PCR amplified cDNA samples were cloned into M13 for sequence analysis. Samples were digested with Pst I and SacI or with Pst I and Bam HI to cut restriction sites in the oligonucleotide primers. Centricon centrifugal microconcentrators (Amicon, 15 Danvers MA) were used to concentrate and desalt the digested PCT products. After transformation in JM101 competent cells, clones containing TCR b inserts were identified by hybridization with a TCR b C-region HRP- labelled probe. Single stranded DNA from positive clones was prepared, and 20 VDJCb sequences determined by the dideoxy chain termination method with an AmpliTaq sequencing kit (Perkin-Elmer).

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Table 7. CDR3 Sequences of TCR Rearrangements Amplified from MS Brains and Controls.

Vb5.2/3	N-D-N-	J		cβ
<b>KL-1</b>				
LCASS	LPGTP	YGYFGSGTRLTVV	(Jb 1.2)	EDLKN
LCASS	LPGTP	YGYTFGSGTRLTVV	(Jb 1.2)	EDLNK
LCASS	LRLAN	SPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	LDRL	YNSPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	QLRLA	NSPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	QLRLA	NSPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	F LG	YNSPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	QPTV	YNNEQFFGQTRLLVL	(Jb 2.1)	EDLKN
LCASS	SDGRM	STQYFGPGTRLLVL	(Jb 2.3)	EDLKN
LCASS	LVAG	SIYEQYFGPGTRLTVT	(Jb 2.7)	EDLKN
LCASS	SEREG	RAQYFGQGTRLTVL	(Jb ?)	EDLKN
LCASS	GGEG	RAQYFGQGTRLTVL	(Jb ?)	EDLKN
<b>KL-3</b>				
LCASS	LDGVP	YGYTFGSGTGTLTVV	(Jb 1.2)	EDLNK
LCASS	LDGVP	YGYTFGSGTRLTVV	(Jb 1.2)	EDLNK
LCASS	LDGV	NYGYTFGSGTRLTVV	(Jb 1.2)	EDLNK
LCASS	LVGRGP	YGYTFGSGTRLTVV	(Jb 1.2)	EDLNK
LCASS	LGGVP	YGYTFGSGTGTLTVV	(Jb 1.2)	EDLNK
LCASS	LRGTP	YGYTFGSGTRLTVV	(Jb 1.2)	EDLNK
LCASS	QPAV	YNEQFFGPGTRLTVL	(Jb 2.1)	EDLKN
LCASS	LELAG	YNEQFFGPGTRLTVL	(Jb 2.1)	EDLKN
LCASS	LGGSEE	DTQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCASS	LGGSE	ETQYFGPGTRLLVL	(Jb 2.5)	EDLKN
LCASS	LGGSV	ETQYFGPGTRLLVL	(Jb 2.5)	EDLKN
LCASS	LGSCTL	QETQYFGPGTRLLVL	(Jb 2.5)	EDLKN
LCASS	LASCTL	QETQYFGPGTRLLVL	(Jb 2.5)	EDLKN
LCASS	LASCTL	QETQYFGPGTRLLVL	(Jb 2.5)	EDLKN
LCASS	PT	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	PT	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	QGS	TFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	L	SGANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	LR	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	LR	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	LVAG	SIYEQYFGPGTRLTVT	(Jb 2.7)	EDLKN
LCASS	LVAG	SIYEQYFGPGTRLTVT	(Jb 2.7)	EDLKN
LCASS	LVAG	SIYEQYFGPGTRLTVT	(Jb 2.7)	EDLKN

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Vb5.2/3	N-D-N-J			cβ
<u>LJ 1</u>				
LCAS	TLRL	GNSPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	DSS	ETQYFGPGTRLLVL	(Jb 2.5)	EDLKN
LCASS	LR	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	LR	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	PT	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	LVAGI	YEQYFGPGTRLTVT	(Jb 2.7)	EDLKN
LCASS	LVAGSI	YEQYFGPSTRLTVT	(Jb 2.7)	EDLKN
LCASS	LVAGSI	YEQYFGPSTRLTVT	(Jb 2.7)	EDLKN
<u>Muscle infiltrating lymphocytes</u>				
LCASS	LGSPGYR	TNEKLFFGSGTQLSVL	(Jb 1.4)	EDLNK
LCASS	FTGAY	YNEQFFGPGTRLTVL	(Jb 2.1)	EDLKN
LCASS	RRTSGFVH	DTQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCAS	ARRTSGFV	TDQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCAS	TARRTSGFV	TDQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCA	TARRTSGFV	TDQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCA	TARRTSGFV	TDQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCA	TARRTSGFV	TDQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCA	TARRTSGFV	TDQYFGPGTRLTVL	(Jb 2.3)	EDLKN
LCAS	RQGART	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
<u>JO (PBLs)</u>				
LCASS	VALQDR	YGYTFGSGTGTLTVV	(Jb 1.2)	EDLNK
LCASS	TVRGS	QPQHFGDGTRLSIL	(Jb 1.5)	EDLNK
LCASS	PGM	KNIQYFGAGTRLSVL	(Jb 2.4)	EDLKN
LCASS	DSPSG	QETQYFGPGTRLTVL	(Jb 2.5)	EDLKN
LCASS	RPGNIR	ETQYFGPGTRLSVL	(Jb 2.5)	EDLNK
LCASS	RSQGART	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
<u>BM (PBLs)</u>				
LCASS	DAG	YNSPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	YRTQL	NSPLHFGNGTRLTVT	(Jb 1.6)	EDLNK
LCASS	LEHRPT	AKNIQYFGAGTRLSVL	(Jb 2.4)	EKLKN
LCASS	PER	GANVLTFGAGSRLTVL	(Jb 2.6)	EDLKN
LCASS	QEA	SYEQYFGPGTRLTVT	(Jb 2.7)	EKLKN
LCAS	RLVRDLSH	EQYFGPSTRLTVT	(Jb 2.7)	EDLKN

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Table 8. Nucleotide sequence Homology in the Use of LeuArgGly and LeuGlyGlyGlu.

Sample	N-D-N-J											
KL3	AGCAGC		CTA	CGC	GGG	GCC	AAC		S	S	LRGAN	(Vb5.2/Jb2.6)
	AGCAGC		TTA	CGC	GGG	ACA	CCC		S		LRGTP	(Vb5.2/Jb1.2)
KL1	AGCAGC		TTG	CGC	TTG	GCT	AAT		SS		LRLAN	(Vb5.2/Jb1.6)
	AGC	CAG	TTG	CGC	TTG	GCT	AAT		S	Q	LRLA	(Vb5.2/Jb1.6)
	AGCAGC	CAG	TTG	CGC	TTG	GCT	AAT		SS	Q	LRLA	(Vb5.2/Jb1.6)
	AGCAGC		TTG	GAT	CGC	TTG	TAT	AAT	SS		LDRLA	(Vb5.2/Jb1.6)
LJ1	AGC	ACG	TTG	CGC	TTG	GGT			S	T	LRLG	(Vb5.2/Jb1.6)
	AGCAGC		CTA	CGG	GGG	GCC	AAC		SS		LRGAN	(Vb5.2/Jb2.6)
	AGCAGC		CTA	CGG	GGG	GCC	AAC		SS		LRGAN	(Vb5.2/Jb2.6)
MS18^	ACGACG		TTG	AGG	GGG	GCG	CTA		SS		LRGAL	(Vb5.2/Jb2.4)
BF1*	AGCAGC		CTC	AGG	GGG				SS		LRG	(Vb6/Jb1.6)
E*	AGCAGC		ATA	AGG	GGA	AGC			SS		IRGS	(Vb6/Jb2.7)
BD3*	AGCAGC		ATC	GTC	AGG	GGA	TCG		SS		IVRGS	(Vb6/Jb2.7)
ph11#	AGCAGT		TTA	AGG	GCG	GGA			SS		LRAG	(Vb8/Jb1.1)
12H6+	AGCAGC		CTC	CGG	GAC	TTT			SS		LRDF	(Vb13/Jb2.1)
KL3	AGCAGC		TTG	GGA	GGG	GTA	CCC	TAT	SS		LGGVPY	(Vb5.2/Jb1.2)
	AGCAGC		TTG	GGA	GGG	TCC	GAA	GAG	SS		LGGSEE	(Vb5.2/Jb2.3)
	AGCAGC		TTG	GGA	GGG	TCC	GAA	GAG	SS		LGGSEE	(Vb5.2/Jb2.5)
	AGCAGC		TTG	GGA	GGG	TCC	GTT	GAG	SS		LGGsVE	(Vb5.2/Jb2.5)
4e	AGCAGC		CTG	GGG	GGC	GAA			SS		LGGE	(Vb8.2/Jb2.5)

- ^ CDR3 usage in human MBP 88-99 specific T cell line (Martin, et al., 1991 J.E.M. 173:19-24)
- \* CDR3 usage in rat spinal cord derived T cell clones specific for BP 85-99 (Gold et al., 1992 J.I. 148:1712-1717)
- @ CDR3 usage in rat lymph node derived T cell clone specific for BP 85-99 (Gold et al., 1992)
- # Cone derived from a human tonsil cDNA library (Tillinghast et al., 1986 Science 233:879-883)
- + Noncytolytic mouse T cell clone specific for the influenza virus strain A/PR8/34 (Morahan et al., 1989)

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Table 9.

HLA CLASS II		Cells/ $\mu$ l	%TCR+CD8-		%TCR+CD8+	
NM	DRB1	DQA1	DQB1	DPB1	Va2	Va2
PATIENTS						
SLC	0101/02,0401	0101,0301	0302,0501	0401,0401	760 530	4.5 2.6 1.3 2.3 6.3 5.7 2.0 1.8 1.3 0.7 0.7 8.0 12.4 0.8
EWP	0101/02,0401	0101,0301	0302,0501	0401,0401	850 650	1.2 2.0 0.7 1.6 6.5 3.9 1.8 1.2 2.5 2.1 0.5 0.8 1.8 0.9
BLM	0301,1301	0103,0501	0201,0603	0201,0402	1500 400	1.9 2.7 1.0 3.0 6.0 4.2 2.1 2.3 3.9 1.1 2.2 2.7 3.1 1.6
NS	0403,06,07	0201,0301	0302,0303	0401,0401	750 370	3.3 2.0 0.8 2.7 1.8 3.2 1.9 3.3 1.4 0.7 1.3 0.5 4.2 0.8
SLB	0101/02,1104	0101,0501	0301,0501	0301,0401	570 270	2.7 3.2 2.4 1.6 6.1 4.7 2.1 2.7 3.1 0.9 1.0 2.3 3.3 1.2
SJP	0101/02,1303	0101,0501	0301,0501	0301,0402	540 290	4.5 3.5 2.4 4.0 4.9 5.0 2.8 3.1 2.0 0.9 1.1 1.1 8.1 1.8
LSH	0103,1001	0101,0101	0501,0501	0401,0401	1200 300	5.1 1.6 0.9 4.8 2.8 4.5 2.3 2.3 1.7 0.5 1.2 0.8 3.4 1.5
GAS	0301,1303	0501,0501	0201,0301	0101,0101	620 270	2.9 3.3 1.5 4.1 4.3 5.4 2.5 2.0 2.4 1.0 1.5 1.6 5.6 1.5
EV	0404,0301	0301,0501	0201,0302	0101,0601	880 280	3.1 2.6 nd 2.9 5.8 5.2 2.2 0.9 1.9 nd 1.1 1.3 3.3 0.8
JDM	07,1503/04	0102,0201	0201,0602	0101,1001	680 470	2.7 2.2 2.4 3.1 3.2 5.4 1.9 2.0 1.3 2.2 1.4 0.8 3.8 1.5
AM	07,1501	0102,0101	0501,0501	0401,0401	560 360	1.6 2.8 1.2 1.6 5.6 3.6 1.2 1.1 2.0 0.8 0.6 1.7 2.8 0.6
LC	0301,1501	0102,0501	0201,0602	0301,0401	990 470	3.8 3.1 1.1 2.7 4.1 5.2 1.7 1.0 1.6 0.6 0.9 1.1 3.4 1.2
CONTROLS						
SZ	1104,1502	0103,0103	0601,0603	0201,1401	610 540	3.1 2.8 1.0 3.6 6.1 3.3 2.2 5.9 3.9 0.6 2.2 3.7 2.5 1.6
MB	1501,-	0101,-	0602,-	0301,-	1300 636	1.6 1.0 5.6 2.6 2.0 2.2 1.2 0.9 1.4 0.2 0.5 0.3 0.4 0.5
IL	07,1501	0102,0201	0201,0602	nd	nd nd	nd 1.0 0.5 nd 2.3 4.9 2.1 nd 1.2 0.6 nd 1.3 3.3 1.5
RL	0801,1501	0102,0401	04,0501	0301,0301	nd nd	nd 1.6 0.7 2.9 2.0 4.8 3.3 nd 1.4 0.4 1.2 0.6 2.4 0.9

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Flow Cytometry: Peripheral blood mononuclear cells (PBMC) were prepared by Ficoll-Hypaque density gradient centrifugation as described (Jackson and Warner, 1985). Briefly, 20 ml of blood at room temperature were diluted with an equal volume of saline, underlayered with Histopaque-1077-1 (Sigma, St. Louis, MO) and centrifuged 30 min. at 400 g. PBMC were washed twice by centrifugation at 250 g for 10 min with staining solution (saline containing 1% fetal calf serum). Three-color staining of 20  $\mu$ l of 2X10<sup>7</sup> PBMC suspensions was performed in 96 well plates (Costar, Cambridge, MA) at 4°C by incubating 20  $\mu$ l of FITC-conjugated Diversi-T ab TCR Screening Panel (T Cell Sciences, Cambridge, MA) monoclonal antibodies, 20  $\mu$ l of phycoerythrin conjugated anti-Leu-3a (CD4) (Becton Dickinson, San Jose, CA) and 20  $\mu$ l of PerCP anti-Leu-2a (CD8) (Becton Dickinson) for 30 min. The PBMC were washed three times in staining solution and fixed with 1% formaldehyde. Fluorescence analysis was carried out on a Becton Dickinson FACScan.

20 II. Prevention of EAE with Competitive Peptides  
Materials and Methods

The determinant of myelin basic protein (MBP) P5-17 contains a pattern P-S-Q-R-H-G-S-K-Y-L-A-T-A. Using an algorithm for the subject motif for predicting T-cell clones, the epitope of the clone F1-28, an MBP-specific T-cell was isolated (Zamvil, et al., J. Exp. Med. (1985) 162:2107), which clone recognizes the autoantigen myelin basic protein. A peptide corresponding to amino acids P35-47 containing two patterns GILDB and RFFS was synthesized and shown to be stimulatory. The peptide determinant tested was GILDSIGRFFSGDRGAP. The stimulatory epitope was shown with overlapping peptides to actually consist of LDSIGRFFSG-DRGAP (Zamvil, et al., Nature (1986) 324:258).

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P35-47 of the human myelin basic protein (MBP) comprising the RFFS motif was found to be stimulatory with mouse T-cells restricted by MHC I-E<sub>a</sub><sup>u</sup>E<sub>b</sub><sup>u</sup>. P5-17 of the human myelin basic protein comprising the motif KYLAT was found to be stimulatory with mouse T-cells restricted by I-A<sub>a</sub><sup>u</sup>A<sub>b</sub><sup>u</sup> or I-A<sub>a</sub><sup>s</sup>A<sub>b</sub><sup>u</sup>; and P89-101 of the human myelin basic protein comprising the motif HFFK was found to be stimulatory with mouse T-cells restricted by I-A<sub>a</sub><sup>s</sup>A<sub>b</sub><sup>s</sup>.

The algorithm can be used to define what immunogenic part of an autoantigen shares sequence homology with pathogens. For example, in the case of MBP P35-47 shared with paramyxoviruses and influenza and MBP 89-101 shared with picornavirus, the critical sequence triggering the T-cell is shared with a pathogen.

In another disease, myasthenia gravis, a T-cell epitope was discovered by using the subject algorithm to construct synthetic peptides of the acetylcholine receptor. The peptide AChR P215-232, DTPYLDITYHFVMQRLPL was particularly stimulatory in a number of myasthenics. Other stimulatory peptides included 277-291 and 330-347 which followed the subject algorithm. Antigen-specific T-cell clones are isolated from peripheral blood lymphocytes (PBL), cultured in vivo with antigen and syngeneic irradiated PBL as antigen presenting cells (APL) (Cunningham, et al., J. Gen. Virol. (1985) 66:249); Eckles, et al., Nature (1981) 301:716).

Synthetic MBP Peptides: Peptides corresponding to the amino acid sequences of rat (R) and bovine (B) MBP (Martenson, 1984, In Experimental Allergic Encephalomyelitis. A Useful Model for Multiple Sclerosis. Alvard, ed. Alan Liss, N.Y.), were synthesized as described previously using solid phase techniques (Erickson and Merrifield, 1976, in The Proteins, Vol. 2, Neurath, ed. Academic Press, NY, p. 255). Peptides were separated from the various organic side products and the purity was determined by high pressure liquid phase column (Merck, Darmstadt, Germany) and by amino acid analysis. These



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peptides were not further purified since they all contained greater than 90% of the desired product. The subject peptides described above were employed in the following test procedure. To render autoimmunogenic peptides tolerigenic, these peptides may be conjugated to lymphocytes (Sriram, et al., 1983, supra) or by coupling the peptide to a carrier such as tetanus toxoid or bovine serum albumin, employing conventional linking groups (Herzenberg, et al., Ann. Rev. Imm. (1983) 1:609-632).

Proliferation Assay: Proliferative responses were determined as described previously (Zamvil, et al., Nature (1985) 317:355).  $1 \times 10^4$  T-cells were cultured with  $5 \times 10^5$  X-irradiated (3,000 rad) PL/J splenic APC in 0.2 ml of culture media in 96 well flat-bottomed microtiter plates (Falcon, 3072). Peptides were added to culture giving the final concentrations indicated. At 48 hours incubation, each well was pulsed with 1  $\mu$ Ci  $^3$ H-thymidine and harvested 16 hours later. The mean c.p.m. thymidine incorporation was calculated for triplicate cultures. Standard deviations from replicate cultures were within 10% mean value.

Development of MBP-specific T cell clones in the rat: T cell lines are selected from LN or SC (spinal cords) of rats immunized with guinea pig myelin basic protein or with MBP peptide 87-99 (200 $\mu$ g) in CFA. Supernatants from ConA-stimulated Lewis rat splenocytes are used as the source of IL-2 to expand Ag-stimulated T cells. T line cells were cloned by limiting dilution or FACS sorting. After cloning subsequent restimulation with antigen was accomplished in 96-well flat bottom plates using  $10^6$  irradiated syngeneic thymocytes/well. After 72 h of stimulation, clones were refed with growth medium and expanded subsequently in 24-well flat-bottom plates. Restimulation in 24-well plates was accomplished by using approximately  $4 \times 10^5$  cloned cells in the presence of  $10^6$  irradiated thymocytes and 25  $\mu$ g peptide.

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Proliferation assays: were performed in 96-well microtiter plates. Briefly,  $2 \times 10^4$  T cells and  $10^6$  irradiated thymocytes/well were incubated with stimulation medium only, Con A, or antigen. The cultures were harvested onto glass fiber filters and TdR uptake was assessed by liquid scintillation. Mean cpm were calculated from triplicate wells. In some experiments, competitor peptides, or anti I-A (OX-6) or anti-I-E antibodies (OX-17) were used to evaluate which MHC molecules were used to restrict the T cell response, or to determine whether peptides could antagonize stimulation by the native peptide.

#### IIa. Prevention of EAE using Synthetic Peptides N1-20 of MBP

A number of peptides were prepared as shown in the following table.

TABLE 10

AcN1-20 AcNHASQKRPSQRHGSKYLATAST

1

10

20

AcN1-11
N2-11
N1-11
N1-20
AcN5-20
AcN9-20
N35-47
N90-101

Antigen presenting cells (APC) were preincubated with various peptides ( $67 \mu\text{M}$ ) as the competitor, for 30 min

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before the addition of AcN1-11 (from 0.067 to 67  $\mu$ M) together with an I-A<sup>u</sup> restricted and AcN1-11 specific T-cell clone, PJR25. In the assay using live APC, the proliferation of PJR25 to AcN1-11 was inhibited in the presence of an excess amount of N1-20 or AcN5-20. Inhibition was evident at the ratio (the competitor:the stimulator) 6:1 or 10:1, and the percentage of inhibition was 79% at 10:1 ( $p > 0.001$  compared to medium control, students test) and 52% at 6:1 ( $p > 0.001$ ) for N1-20 and 50% at 10:1 ( $p > 0.01$ ) for AcN5-20. The inhibitory effect can be reversed by increasing the concentration of AcN1-11 in culture. Peptides N1-11 and AcN2-11 fail to inhibit the response. Also, inhibition is not evident with peptides N35-47 and N90-101, which are restricted exclusively to I-E<sup>u</sup> and I-A<sup>s</sup> molecules. Peptide AcN9-20, which includes a I-A<sup>u</sup> restricted epitope did not inhibit the response in the presence of live APC. When glutaraldehyde-fixed APC were used for the inhibitory proliferative response, AcN9-20 inhibited the response (59%) at 10:1 ( $p > 0.01$  compared to medium control), as AcN5-20 (92%) ( $p > 0.01$ ) and N1-20 (91%) ( $p > 0.001$ ) did, where inhibition was not seen with N1-11, AcN2-11, N35-47 or N90-101.

The inhibitory effect of N1-20 on the proliferation of PJR25 is time dependent. When N1-20 was added in culture 24 h after the addition of AcN1-11, its inhibitory effect was reduced. The APC, which was pre-incubated for 18h with 67  $\mu$ M of N1-20, then extensively washed and fixed with glutaraldehyde, elicited lowered proliferative responses ( $p < 0.05$  relative to medium control) in the clone than the fixed APC pre-incubated with control peptide AcN2-11 on medium alone. These data support the fact that inhibition occurred at the level of antigen presentation by APC and exclude the possibility that inhibition in vitro might be due to non-specific cytotoxicity. Inhibition was observed not only in the response of PJR25, it was also observed in the response for another AcN1-11 specific clone, R2.2, whose

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- T-cell receptor genes are rearranged differently from that of PJR25, and in the response of primary lymphocytes sensitized with AcN1-11. In contrast, N1-20 did not inhibit the I-A<sup>s</sup> restricted response of a clone, 3r to N90-101.
- 5 Similar competition in vitro was observed not only in clonal populations, but also in AcN1-11 primed heterogeneous populations.

TABLE 11  
PREVENTION OF EAE IN PLSJF<sub>1</sub> AND SJL/J MICE WITH THE COMPETITOR PEPTIDES

Encephalitogen	Mice	Competitor (nmol)	Incidence of EAE	Day of Onset	Severity
AcNI-11 (100)	PLSJF <sub>1</sub>	medium	6/11	13.5 ± 0.5	3.1 ± 1.1
AcNI-11 (100)	PLSJF <sub>1</sub>	AcN2-11 (600)	8/12	13.6 ± 1.0	3.0 ± 1.2
AcNI-11 (100)	PLSJF <sub>1</sub>	N1-20 (600)	0/10*	-	-
AcNI-11 (100)	PLSJF <sub>1</sub>	medium	10/19	15.1 ± 1.7	2.9 ± 1.2
AcNI-11 (100)	PLSJF <sub>1</sub>	N1-20 (300)	1/16*	11.0	2.0
AcNI-11 (100)	PLSJF <sub>1</sub>	medium	5/11	17.4 ± 0.5	3.8 ± 1.6
AcNI-11 (100)	PLSJF <sub>1</sub>	AcN9-20 (600)	1/12*	17.0	4.0
AcNI-11 (100)	PLSJF <sub>1</sub>	medium	7/12	15.0 ± 1.5	3.1 ± 0.6
AcNI-11 (100)	PLSJF <sub>1</sub>	AcN9-20 (300)	0/10*	-	-
N89-101 (200)	SJL/J	medium	3/14	12.0	2.0 ± 1.7
N89-101 (200)	SJL/J	N1-20 (1000)	3/9	12.6 ± 0.6	3.6 ± 0.6
N89-101 (200)	SJL/J	medium	4/11	15.2 ± 2.1	2.2 ± 1.2
N89-101 (200)	SJL/J	N1-20 (600)	5/8	15.8 ± 1.6	3.0 ± 1.0

\* indicates that values are significant at values  $p < 0.01$  chi-square or Fisher's exact test.  
Incidence of EAE was expressed as number of mice with clinical EAE/number of mice immunized; day of onset as mean day of onset ± SD; severity as mean severity of sick mice ± SD. For the induction of EAE, MBP peptide AcN1-11 (100 nmol) or N90-101 (200 nmol) was dissolved in phosphate buffered saline (PBS) and emulsified with complete Freund's adjuvant (CFA) in a 1:1 mixture of PBS and CFA containing H37Ra. Mice were injected with 0.2 ml emulsion at the base of the tail. On the same day and 48 h later, pertussis toxin (List Chemicals, Campbell, CA) was injected intravenously. Mice were examined daily for signs of EAE and assessed for clinical severity, graded from 1 to 5 described before (Zamvil, *et al.*, 1986, *supra*). Some animals were sacrificed 23 to 27 days after immunization for histological examination. For prevention of EAE, animals were immunized with AcN1-11 (100 nmol) N89-101 (200 nmol) and medium, or in a mixture with the competitor peptide (300, 600 or 1000 nmol).

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As shown in the above table, AcN2-11 could not prevent disease even if the ratio is 6:1 (the competitor AcN1-11), while injection of N1-20 significantly ( $p < 0.01$  Fisher's exact test) prevented the clinical development of EAE at the ratio of 3:1 ( $p < 0.001$ ). In addition, AcN9-20 had a preventative effect on EAE at the ratio of 3:1 ( $p < 0.001$ ) and 6:1 ( $p < 0.001$ ). Injection of N1-20 could not prevent EAE induced with the I-A<sup>s</sup> restricted peptide N89-101 SJL/J mice at the ratio 3:1 or 5:1. In reviewing representative sections of 20 mice treated with competitors (N1-20 and AcN9-20), which did not show any clinical signs of EAE, no perivascular cuffs of submeningeal cell infiltrates were evident.

#### IIb. Binding Specificity of Synthetic Peptides 89-99 of MBP

We made a set of substituted peptides on the sequence VHFFKNIVTPRTP, which is identical in rats, mice and human myelin basic protein (MBP), and corresponds to the I-E restricted epitope MBP 89-99 in the rat. The peptides are shown in Table 12. We measured MHC binding in a FACS assay described in Smilek et al, 1991, Gautam et al 1992a&b.

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Table 12

Set of Alanine Substituted Peptides for Myelin Basic  
Protein Epitope in Rat and Man

	89	90	91	92	93	94	95	96	97	98	99	100	101
5	V	H	F	F	K	N	I	V	T	P	R	T	P
	A												ala 1
		A											ala 2
			A										ala 3
				A									ala 4
					A								ala 5
						A							ala 6
							A						ala 7
								A					ala 8
									A				ala 9
										A			ala 10
											A		ala 11
												A	ala 12
													A ala 13

The numbering was chosen so that our results can be directly compared to the studies of Martin et al, 1992 on the human MBP sequence, which is identical, but numbered differently due to a deletion near the N-terminus of the myelin basic protein molecule in rodents.

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We tested these peptides for their ability to cause EAE when mixed in CFA. As can be seen in Table 13 column 2, below, the peptides Ala4, Ala5, Ala6, Ala8, and Ala10 were incapable of causing EAE, while Ala9 induced EAE in only 1/6 rats. It was seen that Ala4, Ala6, Ala7, and Ala8 are poor binders to I-E, implying that these residues 4F,6N,7I, and 8V are critical in MHC binding. The peptides Ala6 and Ala8 weakly stimulate an encephalitogenic T cell line raised against native peptide (89-101). When tested on MS18 the human cytotoxic T cell line which is V $\beta$ 5.2LRGA, and which recognizes 87-106, the Ala6 and Ala8 peptides cannot serve as targets for cytotoxicity with HLADRB1\*1501 targets. Ala8 can compete with 87-106 to block cytotoxicity of MS18. In the Lewis rat both Ala6 and Ala8 can block proliferation of an 89-101 T cell line when given competitively with native peptide in vitro. Despite their inability to bind well to I-E, peptides ala6 and ala8 block the development of EAE when mixed with native peptide in a 5:1 molar ratio with CFA (0/6 sick with native plus ala6, 2/6 with native plus ala8, compared to 11/12 with native peptide alone). These competitor peptides Ala6 and Ala8, though poor I-E binders can apparently compete with native peptide and antagonize the T cell receptor.



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**Table 13:**  
Summary of Assays With Substituted Peptides In Rat# and in MS Patient\*

peptide	EAE	IC50 I-E Binding	CTL	Prolif.	CTL	Comp Assay Prolif	EAE
native	21/21	14 $\mu$ M	cytotoxic	4+	negative	MINUS	11/12
Ala 1	6/6	31	ND	4+	ND	ND	ND
Ala 2	6/6	14	ND	3+	ND	ND	ND
Ala 3	6/6	11	cytotoxic	3+	ND	ND	ND
Ala 4	0/6	>200	cytotoxic	negative	$\pm$	ND	ND
Ala 5	0/6	21	negative	negative	ND	ND	ND
Ala 6	0/21	>200	negative	1+	ND	plus	0/6
Ala 7	6/6	>200	negative	2+	plus	ND	ND
Ala 8	1/15	>200	negative	1+	plus	plus	2/6
Ala 9	1/6	14	cytotoxic	negative	ND	ND	ND
Ala 10	0/6	14	cytotoxic	1+	ND	ND	ND
Ala 11	4/6	20	cytotoxic	negative	ND	ND	ND
Ala 12	6/6	14	cytotoxic	1+	ND	ND	ND
Ala 13	3/6	10	ND	2+	ND	ND	ND

\*Data from Martin et al, 1992 with MS18 a CD4+cytotoxic T cell clone specific for MBP 87-106 restricted by HLA DRB1\*1501 DQB1\*0602 DPB1\*0401, utilizing VB5.2 with LRGA in the (N)D(N), CDR3 region.

Data shown above demonstrate formulations which are weak MHC binders which nevertheless antagonize TCR recognition of MBP peptide 87-99, and which prevent EAE when mixed with MBP in complete Freund's adjuvant in a 5:1 molar ratio. In addition Ala9 and Ala10 are good MHC binders yet are nonencephalitogenic and are weak stimulators of an encephalitogenic T cell line induced with native 87-99

The compound Ala4 is a weaker binder to I-E ( $IC_{50} > 200$  mM), and does not stimulate an 87-99 T cell line, and does not cause EAE. These compounds may antagonize TCR recognition of native 87-99 as well.

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Data shown above demonstrate formulations which are weak MHC binders which nevertheless antagonize TCR recognition of MBP peptide 87-99, and which prevent EAE when mixed with MBP in complete Freund's adjuvant in a 5:1 molar ratio. In addition Ala9 and Ala10 are good MHC binders yet are nonencephalitogenic and are weak stimulators of an encephalitogenic T cell line induced with native 87-99.

The compound Ala4 is a weaker binder to I-E ( $IC_{50} > 200$  mM), and does not stimulate an 87-99 T cell line, and does not cause EAE. These compounds may antagonize TCR recognition of native 87-99 as well.

IIC. Prevention of EAE with Synthetic Peptides 87-99 of MBP

In the following experiment we demonstrate the possibility of preventing EAE, by co-immunizing with MBP and a poor MHC binder, like Ala6 or Ala8, with the capacity to block an 87-99 response.

Table 14

Competition with Ala6 and Ala5 For Prevention of EAE With MBP87-99

<u>MBP 87-99</u>	<u>ala 6</u>	<u>ala 8</u>	<u>MBP Acl-20</u>	<u>Incidence of EAE</u>
0.2 mg				11/12
	0.2 mg			0/6
		0.2 mg		0/6
			0.2 mg	0/6

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			1 mg	0/6
0.2 mg			1 mg	6/6
0.2 mg	1 mg			0/6
0.2 mg		1 mg		2/6

Incidence of EAE was expressed as number of mice with clinical EAE/number of mice immunized. For the induction of EAE, MBP peptide was dissolved in phosphate buffered saline (PBS) and emulsified with complete Freund's adjuvant (CFA) in a 1:1 mixture of PBS and CFA. Mice were injected with 0.2 ml emulsion at the base of the tail. On the same day and 48 h later, pertussis toxin (List Chemicals, Campbell, CA) was injected intravenously. Mice were examined daily for signs of EAE. For prevention of EAE, animals were immunized with MBP 87-99 (0.2 mg), or in a mixture with the competitor peptide (1 mg).

As shown in the above table, AcN1-20 could not prevent disease caused by N87-99. Co-injection of Ala 6 completely prevented the clinical development of EAE. In addition, Ala 8 had a preventative effect on EAE. This is the first example of a TCR antagonist which can block the CDR3 region of the TCR involved in multiple sclerosis.

It is evident from the above results, by modification of a peptide, particularly an internal peptide of a larger peptide that combines to an MHC and is associated with an autoimmune disease or other immune attack on mammalian cells, particularly syngeneic cells, the host may be protected from the immune attack. Thus, as T-cell immunodominant sequences are identified, these sequences may be modified by modifying the amino acid sequence to produce antagonists to the autoimmune disease.

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It is evident from the above results that the subject method can be used for identifying T-cell receptors associated with degenerative disorders. Thus, by a simple screening technique, one may identify those T-cell receptors which cause or combat disease and by various procedures inhibit or enhance their activity. The subject invention provides the capability to diagnose individuals susceptible to degenerative diseases associated with T-cell receptor variable regions. By screening degenerative tissue for T-cell receptors and identifying the specific T-cell receptors associated with that tissue and the HLA of the particular host, the relationship between the T-cell receptor, HLA and the disease may be established. Contrastingly, when the T-cells are associated with combatting a neoproliferative disorder, the particular T-cells may be employed for prophylaxis or therapy.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Steinman, Lawrence  
Oksenberg, Jorge  
Bernard, Claude  
Zamvil, Scott  
Mitchell, Dennis J  
Karin, Nathan
- (ii) TITLE OF INVENTION: INTERACTION OF T CELL RECEPTORS AND  
ANTIGEN IN AUTOIMMUNE DISEASE
- (iii) NUMBER OF SEQUENCES: 177
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: FLEHR, HOHBACH, TEST, ALBRITTON & HERBERT
  - (B) STREET: 4 Embarcadero Center, Suite 3400
  - (C) CITY: San Francisco
  - (D) STATE: California
  - (E) COUNTRY: USA
  - (F) ZIP: 94111-4187
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: PCT/US94/
  - (B) FILING DATE: 22-SEP-1994
  - (C) CLASSIFICATION:
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  - (B) FILING DATE: 22-SEP-1993
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Rowland, Bertram I
  - (B) REGISTRATION NUMBER: 20,015
  - (C) REFERENCE/DOCKET NUMBER: FP55875-4BIR
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (415) 781-1989
  - (B) TELEFAX: (415) 398-3249
  - (C) TELEX: 910 277299

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 57 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGTACCGAG ATGACGAACC CACCTTTGGG ACAGGCACTC AGCTAAAAGT GCAACTC

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## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu Cys Ala Ser Ser  
1 5

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCCTACGCA GCTTGCGCAG CCTGCGGTTG CGC

33

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTGCCCTGAG AGATGCCAGA G

21

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGTTCCCGAG AGGGAGCCAT TGCC

24

## (2) INFORMATION FOR SEQ ID NO:6:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTGAACAGT CAACAGGGAG A

21

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACAAGCATT A CTGTACTCCT A

21

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCCCTGAAC ATTCAGGA

18

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTCACCTTCT AGCCTGCTGA

20

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid

-70-

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGGAGCCATT GTCCAGATAA A

21

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGAGAGAATG TGGAGCAGCA TC

22

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATCTCAGTGC TTGTGATAAT A

21

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCCAGCTGG TGGAGCAGAG CCCT

24

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear



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(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGAAAGCAAG GACCAAGTGT T

21

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGAAGGTAA CTCAAGCGCA GACT

24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTTATGAGA ACACTGCGT

19

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCAGCTTCCC TTCCAGCAAT

20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:  
AGAACCTGAC TGCCCAGGAA 20
- (2) INFORMATION FOR SEQ ID NO:19:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:  
CATCTCCATG GACTCATATG A 21
- (2) INFORMATION FOR SEQ ID NO:20:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:  
GACTATACTA ACAGCATGT 19
- (2) INFORMATION FOR SEQ ID NO:21:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:  
TGTCAGGCAA TGACAAGG 18
- (2) INFORMATION FOR SEQ ID NO:22:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:  
AATAGGTCGA CACACTTGTC ACTGGA 26

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## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ACGAAGACGG ACCACCGCCC TG

22

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CACGTTGTGG GTGACGCCGT C

21

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CAGAAGGTAA CTGCAGCGCA GACT

24

## (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTGGGGATCC AGAGCACAGA AGTATACTGC

30

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:

-74-

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CAGAACCCTG ACCCTGCCGT GTAC

24

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTGTCCACAG TTTAGGTTTCG TATCTGT

27

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTGGAGCTCC TGTAAGGA G

21

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAGAACCCTG ACCCTGCCGT GTAC

24

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCACAACAGT TCCCTGACTT GCAC

24

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCATCAACCA TGCAAGCCTG ACCT

24

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTCTCTAGAG AGAAGAAGGA GCGC

24

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACGATCCAGT GTCAAGTCGT

20

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35: 22  
ATACTTCAGT GAGACACAGA GA
- (2) INFORMATION FOR SEQ ID NO:36:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36: 23  
TTCCCTAACT ATAGCTCTGG CTG
- (2) INFORMATION FOR SEQ ID NO:37:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37: 20  
AGGCCTGAGG GATCCGTCTC
- (2) INFORMATION FOR SEQ ID NO:38:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38: 22  
CCTGAATGCC CCAACAGCTC TC
- (2) INFORMATION FOR SEQ ID NO:39:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

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ACTTTAACAA CAACGTTCCG A

21

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CTAAATCTCC AGACAAAGCT CAC

23

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCCAAAACT CATCCTGTAC CT

22

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TGTTCTCAAA CCATGGGCCA TGAC

24

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GATACTGACA AAGGAGAAGT CTCAGAT

27

(2) INFORMATION FOR SEQ ID NO:44:

-78-

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GGTGAGGGTA CAACTGCC

18

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

ACCCAAGATA CCTCATCACA G

21

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

AGTGTCTCTC GACAGGCACA G

21

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CATGATAATC TTTATCGACG TGTT

24

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid



-79-

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

AGCCCAATGA AAGGAACACA GTCAT

25

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

AGCCCAATGA AAGGACACAG TCAT

24

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ACCCCGAAA AAGGACATAC T

21

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CTCTGAGGTG CCCAGAA

18

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

-80-

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TTCTGATGGC TCAAACAG

18

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Leu	Cys	Ala	Ser	Ser	Leu	Pro	Gly	Thr	Pro	Tyr	Gly	Tyr	Phe	Gly	Ser
1				5					10					15	
Gly Thr Arg Leu Thr Val Val Glu Asp Leu Lys Asn															
20 25															

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Leu	Cys	Ala	Ser	Ser	Leu	Pro	Gly	Thr	Pro	Tyr	Gly	Tyr	Thr	Phe	Gly
1				5					10					15	
Ser Gly Thr Arg Leu Thr Val Val Glu Asp Leu Asn Lys															
20 25															

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Leu	Cys	Ala	Ser	Ser	Leu	Arg	Leu	Ala	Asn	Ser	Pro	Leu	His	Phe	Gly
1				5					10					15	
Asn Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys															
20 25															

-81-

## (2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Leu Cys Ala Ser Ser Leu Asp Arg Leu Tyr Asn Ser Pro Leu His Phe  
1 5 10 15  
Gly Asn Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys  
20 25 30

## (2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Leu Cys Ala Ser Gln Leu Arg Leu Ala Asn Ser Pro Leu His Phe Gly  
1 5 10 15  
Asn Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys  
20 25

## (2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Leu Cys Ala Ser Ser Gln Leu Arg Leu Ala Asn Ser Pro Leu His Phe  
1 5 10 15  
Gly Asn Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys  
20 25 30

## (2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Leu	Cys	Ala	Ser	Ser	Phe	Leu	Gly	Tyr	Asn	Ser	Pro	Leu	His	Phe	Gly
1				5					10					15	
Asn	Gly	Thr	Arg	Leu	Thr	Val	Thr	Glu	Asp	Leu	Asn	Lys			
			20					25							

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Leu	Cys	Ala	Ser	Ser	Gln	Pro	Thr	Val	Tyr	Asn	Asn	Glu	Gln	Phe	Phe
1				5					10					15	
Gly	Gln	Arg	Thr	Arg	Leu	Leu	Val	Leu	Glu	Asp	Leu	Lys	Asn		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Leu	Cys	Ala	Ser	Ser	Ser	Asp	Gly	Arg	Met	Ser	Thr	Gln	Tyr	Phe	Gly
1				5					10					15	
Pro	Gly	Thr	Arg	Leu	Leu	Val	Leu	Glu	Asp	Leu	Lys	Asn			
			20					25							

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Leu	Cys	Ala	Ser	Ser	Leu	Val	Ala	Gly	Ser	Ile	Tyr	Glu	Gln	Tyr	Phe
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

1                      5                                  10    15  
Gly Pro Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
                      20                                  25    30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Ser Glu Arg Glu Gly Arg Ala Gln Tyr Phe Gly  
1 5 10 15  
Gln Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Gly Gly Glu Gly Arg Ala Gln Tyr Phe Gly Gln  
1 5 10 15  
Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Leu Asp Gly Val Pro Tyr Gly Tyr Thr Phe Gly  
1 5 10 15  
Ser Gly Thr Gly Leu Thr Val Val Glu Asp Leu Asn Lys  
20 25

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- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Leu Cys Ala Ser Ser Leu Asp Gly Val Pro Tyr Gly Tyr Thr Phe Gly  
1 5 10 15  
Ser Gly Thr Arg Leu Thr Val Val Glu Asp Leu Asn Lys  
20 25

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Leu Cys Ala Ser Ser Leu Asp Gly Val Asn Tyr Gly Tyr Thr Phe Gly  
1 5 10 15  
Ser Gly Thr Arg Leu Thr Val Val Glu Asp Leu Asn Lys  
20 25

(2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Leu Cys Ala Ser Ser Leu Val Gly Arg Gly Pro Tyr Gly Tyr Thr Phe  
1 5 10 15  
Gly Ser Gly Thr Arg Leu Thr Val Val Glu Asp Leu Asn Lys  
20 25 30

(2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Leu Cys Ala Ser Ser Leu Gly Gly Val Pro Tyr Gly Tyr Thr Phe Gly  
1 5 10 15  
Ser Gly Thr Gly Leu Thr Val Val Glu Asp Leu Asn Lys  
20 25

## (2) INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Leu Cys Ala Ser Ser Leu Arg Gly Thr Pro Tyr Gly Tyr Thr Phe Gly  
1 5 10 15  
Ser Gly Thr Arg Leu Thr Val Val Glu Asp Leu Asn Lys  
20 25

## 0(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Leu Cys Ala Ser Ser Gln Pro Ala Val Tyr Asn Glu Gln Phe Phe Gly  
1 5 10 15  
Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

## (2) INFORMATION FOR SEQ ID NO:72:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Leu Cys Ala Ser Ser Leu Glu Leu Ala Gly Tyr Asn Glu Gln Phe Phe  
1 5 10 15

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Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Leu Cys Ala Ser Ser Leu Gly Gly Ser Glu Glu Asp Thr Gln Tyr Phe  
 1 5 10 15  
 Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Leu Cys Ala Ser Ser Leu Gly Gly Ser Glu Glu Thr Gln Tyr Phe Gly  
 1 5 10 15  
 Pro Gly Thr Arg Leu Leu Val Leu Glu Asp Leu Lys Asn  
 20 25

## (2) INFORMATION FOR SEQ ID NO:75:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Leu Cys Ala Ser Ser Leu Gly Gly Ser Val Glu Thr Gln Tyr Phe Gly  
 1 5 10 15  
 Pro Gly Thr Arg Leu Leu Val Leu Glu Asp Leu Lys Asn  
 20 25

## (2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 31 amino acids



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- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Leu Cys Ala Ser Ser Leu Ala Ser Gly Thr Leu Gln Glu Thr Gln Tyr  
1                      5                      10                      15  
Phe Gly Pro Gly Thr Arg Leu Leu Val Leu Glu Asp Leu Lys Asn  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO:77:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 31 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Leu Cys Ala Ser Ser Leu Ala Ser Gly Thr Leu Gln Glu Thr Gln Tyr  
1                      5                      10                      15  
Phe Gly Pro Gly Thr Arg Leu Leu Val Leu Glu Asp Leu Lys Asn  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 31 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Leu Cys Ala Ser Ser Leu Ala Ser Gly Thr Leu Gln Glu Thr Gln Tyr  
1                      5                      10                      15  
Phe Gly Pro Gly Thr Arg Leu Leu Val Leu Glu Asp Leu Lys Asn  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO:79:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Leu Cys Ala Ser Ser Pro Thr Gly Ala Asn Val Leu Thr Phe Gly Ala  
1 5 10 15  
Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

## (2) INFORMATION FOR SEQ ID NO:80:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Leu Cys Ala Ser Ser Pro Thr Gly Ala Asn Val Leu Thr Phe Gly Ala  
1 5 10 15  
Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

## (2) INFORMATION FOR SEQ ID NO:81:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Leu Cys Ala Ser Ser Gln Gly Ser Thr Phe Gly Ala Gly Ser Arg Leu  
1 5 10 15  
Thr Val Leu Glu Asp Leu Lys Asn  
20

## (2) INFORMATION FOR SEQ ID NO:82:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Leu Cys Ala Ser Ser Ser Gly Ala Asn Val Leu Thr Phe Gly Ala Gly  
1 5 10 15  
Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Leu Gly Ala Asn Val Leu Thr Phe Gly Ala Gly  
1 5 10 15  
Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Leu Arg Gly Ala Asn Val Leu Thr Phe Gly Ala  
1 5 10 15  
Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Leu Val Ala Gly Ser Ile Tyr Glu Gln Tyr Phe  
1 5 10 15  
Gly Pro Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
20 25 30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Leu Cys Ala Ser Ser Leu Val Ala Gly Ser Ile Tyr Glu Gln Tyr Phe  
1 5 10 15  
Gly Pro Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
20 25 30

(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Leu Cys Ala Ser Ser Leu Val Ala Gly Ser Ile Tyr Glu Gln Tyr Phe  
1 5 10 15  
Gly Pro Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
20 25 30

(2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Leu Cys Ala Ser Thr Leu Arg Leu Gly Asn Ser Pro Leu His Phe Gly  
1 5 10 15  
Asn Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys  
20 25

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Leu Cys Ala Ser Ser Asp Ser Ser Glu Thr Gln Tyr Phe Gly Pro Gly

1	5	10	15
Thr	Arg	Leu	Leu Val Leu Glu Asp Leu Lys Asn
	20	25	

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Leu Arg Gly Ala Asn Val Leu Thr Phe Gly Ala  
1 5 10 15  
Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Leu Arg Gly Ala Asn Val Leu Thr Phe Gly Ala  
1 5 10 15  
Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Pro Thr Gly Ala Asn Val Leu Thr Phe Gly Ala  
1 5 10 15  
Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25

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- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Leu Cys Ala Ser Ser Leu Val Ala Gly Ile Tyr Glu Gln Tyr Phe Gly  
1 5 10 15

Pro Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
20 25

(2) INFORMATION FOR SEQ ID NO:94:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Leu Cys Ala Ser Ser Leu Val Ala Gly Ser Ile Tyr Glu Gln Tyr Phe  
1 5 10 15

Gly Pro Ser Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
20 25 30

(2) INFORMATION FOR SEQ ID NO:95:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Leu Cys Ala Ser Ser Leu Val Ala Gly Ser Ile Tyr Glu Gln Tyr Phe  
1 5 10 15

Gly Pro Ser Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
20 25 30

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

Leu Cys Ala Ser Ser Leu Gly Ser Pro Gly Tyr Arg Thr Asn Glu Lys  
1 5 10 15  
Leu Phe Phe Gly Ser Gly Thr Gln Leu Ser Val Leu Glu Asp Leu Asn  
20 25 30  
Lys

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Phe Thr Gly Ala Tyr Tyr Asn Glu Gln Phe Phe  
1 5 10 15  
Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25 30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Leu Cys Ala Ser Ser Arg Arg Thr Ser Gly Phe Val His Asp Thr Gln  
1 5 10 15  
Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25 30

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

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Leu Cys Ala Ser Ala Arg Arg Thr Ser Gly Phe Val Thr Asp Thr Gln  
 1 5 10 15  
 Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:100:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 33 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Leu Cys Ala Ser Thr Ala Arg Arg Thr Ser Gly Phe Val Thr Asp Thr  
 1 5 10 15  
 Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys  
 20 25 30  
 Asn

## (2) INFORMATION FOR SEQ ID NO:101:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Leu Cys Ala Thr Ala Arg Arg Thr Ser Gly Phe Val Thr Asp Thr Gln  
 1 5 10 15  
 Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:102:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

Leu Cys Ala Thr Ala Arg Arg Thr Ser Gly Phe Val Thr Asp Thr Gln  
 1 5 10 15



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Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
                   20                                  25                                  30

## (2) INFORMATION FOR SEQ ID NO:103:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Leu Cys Ala Thr Ala Arg Arg Thr Ser Gly Phe Val Thr Asp Thr Gln  
   1                  5                                  10                                  15

Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
                   20                                  25                                  30

## (2) INFORMATION FOR SEQ ID NO:104:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Leu Cys Ala Thr Ala Arg Arg Thr Ser Gly Phe Val Thr Asp Thr Gln  
   1                  5                                  10                                  15

Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
                   20                                  25                                  30

## (2) INFORMATION FOR SEQ ID NO:105:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 31 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

Leu Cys Ala Ser Arg Gln Gly Ala Arg Thr Gly Ala Asn Val Leu Thr  
   1                  5                                  10                                  15

Phe Gly Ala Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
                   20                                  25                                  30

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## (2) INFORMATION FOR SEQ ID NO:106:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

Leu Cys Ala Ser Ser Val Ala Leu Gln Asp Arg Tyr Gly Tyr Thr Phe  
1 5 10 15  
Gly Ser Gly Thr Gly Leu Thr Val Val Glu Asp Leu Asn Lys  
20 25 30

## (2) INFORMATION FOR SEQ ID NO:107:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

Leu Cys Ala Ser Ser Thr Val Arg Gly Ser Gln Pro Gln His Phe Gly  
1 5 10 15  
Asp Gly Thr Arg Leu Ser Ile Leu Glu Asp Leu Asn Lys  
20 25

## (2) INFORMATION FOR SEQ ID NO:108:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

Leu Cys Ala Ser Ser Pro Gly Met Lys Asn Ile Gln Tyr Phe Gly Ala  
1 5 10 15  
Gly Thr Arg Leu Ser Val Leu Glu Asp Leu Lys Asn  
20 25

## (2) INFORMATION FOR SEQ ID NO:109:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Leu Cys Ala Ser Ser Asp Ser Pro Ser Gly Gln Glu Thr Gln Tyr Phe  
1 5 10 15  
Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
20 25 30

## (2) INFORMATION FOR SEQ ID NO:110:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

Leu Cys Ala Ser Ser Arg Pro Gly Asn Ile Arg Glu Thr Gln Tyr Phe  
1 5 10 15  
Gly Pro Gly Thr Arg Leu Ser Val Leu Glu Asp Leu Asn Lys  
20 25 30

## (2) INFORMATION FOR SEQ ID NO:111:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

Leu Cys Ala Ser Ser Arg Ser Gln Gly Ala Arg Thr Gly Ala Asn Val  
1 5 10 15  
Leu Thr Phe Gly Ala Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys  
20 25 30  
Asn

## (2) INFORMATION FOR SEQ ID NO:112:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

-98-

Leu Cys Ala Ser Ser Asp Ala Gly Tyr Asn Ser Pro Leu His Phe Gly  
 1 5 10 15  
 Asn Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys  
 20 25

## (2) INFORMATION FOR SEQ ID NO:113:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

Leu Cys Ala Ser Ser Tyr Arg Thr Gln Leu Asn Ser Pro Leu His Phe  
 1 5 10 15  
 Gly Asn Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:114:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

Leu Cys Ala Ser Ser Leu Glu His Arg Pro Thr Ala Lys Asn Ile Gln  
 1 5 10 15  
 Tyr Phe Gly Ala Gly Thr Arg Leu Ser Val Leu Glu Lys Leu Lys Asn  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:115:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

Leu Cys Ala Ser Ser Pro Glu Arg Gly Ala Asn Val Leu Thr Phe Gly  
 1 5 10 15  
 Ala Gly Ser Arg Leu Thr Val Leu Glu Asp Leu Lys Asn  
 20 25

## (2) INFORMATION FOR SEQ ID NO:116:

-99-

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

Leu Cys Ala Ser Ser Gln Glu Ala Ser Tyr Glu Gln Tyr Phe Gly Pro  
1                      5                      10                      15  
Gly Thr Arg Leu Thr Val Thr Glu Lys Leu Lys Asn  
                    20                      25

(2) INFORMATION FOR SEQ ID NO:117:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

Leu Cys Ala Ser Arg Leu Val Arg Asp Leu Ser His Glu Gln Tyr Phe  
1                      5                      10                      15  
Gly Pro Ser Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO:118:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

AGCAGCCTAC GCGGGGCCAA C

21

(2) INFORMATION FOR SEQ ID NO:119:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

-100-

AGCAGCTTAC GCGGGACACC C

21

## (2) INFORMATION FOR SEQ ID NO:120:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

AGCAGCTTGC GCTTGGCTAA T

21

## (2) INFORMATION FOR SEQ ID NO:121:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

AGCCAGTTGC GCTTGGCTAA T

21

## (2) INFORMATION FOR SEQ ID NO:122:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

AGCAGCCAGT TGCGCTTGGC TAAT

24

## (2) INFORMATION FOR SEQ ID NO:123:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

AGCAGCTTGG ATCGCTTGTA TAAT

24

## (2) INFORMATION FOR SEQ ID NO:124:

-101-

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

AGCACGTTGC GCTTGGGT

18

(2) INFORMATION FOR SEQ ID NO:125:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

AGCAGCCTAC GGGGGGCCAA C

21

(2) INFORMATION FOR SEQ ID NO:126:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

AGCAGCCTAC GGGGGGCCAA C

21

(2) INFORMATION FOR SEQ ID NO:127:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

ACGACGTTGA GGGGGGCGCT A

21

(2) INFORMATION FOR SEQ ID NO:128:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 base pairs
  - (B) TYPE: nucleic acid

-102-

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

AGCAGCCTCA GGGGG

15

(2) INFORMATION FOR SEQ ID NO:129:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

AGCAGCATAA GGGGAAGC

18

(2) INFORMATION FOR SEQ ID NO:130:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

AGCAGCATCG TCAGGGGATC G

21

(2) INFORMATION FOR SEQ ID NO:131:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

AGCAGTTTAA GGGCGGGA

18

(2) INFORMATION FOR SEQ ID NO:132:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear



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(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

AGCAGCCTCC GGGACTTT

18

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

AGCAGCTTGG GAGGGGTACC CTAT

24

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

AGCAGCTTGG GAGGGTCCGA AGAG

24

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

AGCAGCTTGG GAGGGTCCGA AGAG

24

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

AGCAGCTTGG GAGGGTCCGT TGAG

24

(2) INFORMATION FOR SEQ ID NO:137:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

AGCAGCCTGG GGGGCGAA

18

(2) INFORMATION FOR SEQ ID NO:138:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 7 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

Ser	Ser	Leu	Arg	Gly	Ala	Asn
1				5		

(2) INFORMATION FOR SEQ ID NO:139:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

Ser	Leu	Arg	Gly	Thr	Pro
1				5	

(2) INFORMATION FOR SEQ ID NO:140:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 7 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

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Ser Ser Leu Arg Leu Ala Asn  
1 5

## (2) INFORMATION FOR SEQ ID NO:141:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

Ser Gln Leu Arg Leu Ala  
1 5

## (2) INFORMATION FOR SEQ ID NO:142:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

Ser Ser Gln Leu Arg Leu Ala  
1 5

## (2) INFORMATION FOR SEQ ID NO:143:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

Ser Ser Leu Asp Arg Leu Ala  
1 5

## (2) INFORMATION FOR SEQ ID NO:144:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

-106-

Ser Thr Leu Arg Leu Gly  
1 5

(2) INFORMATION FOR SEQ ID NO:145:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:  
Ser Ser Leu Arg Gly Ala Asn  
1 5

(2) INFORMATION FOR SEQ ID NO:146:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:  
Ser Ser Leu Arg Gly Ala Asn  
1 5

(2) INFORMATION FOR SEQ ID NO:147:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:  
Ser Ser Leu Arg Gly Ala Leu  
1 5

(2) INFORMATION FOR SEQ ID NO:148:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

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Ser Ser Leu Arg Gly  
1 5

## (2) INFORMATION FOR SEQ ID NO:149:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

Ser Ser Ile Arg Gly Ser  
1 5

## (2) INFORMATION FOR SEQ ID NO:150:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

Ser Ser Ile Val Arg Gly Ser  
1 5

## (2) INFORMATION FOR SEQ ID NO:151:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

Ser Ser Leu Arg Ala Gly  
1 5

## (2) INFORMATION FOR SEQ ID NO:152:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

Ser Ser Leu Arg Asp Phe  
1 5

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

Ser Ser Leu Gly Gly Val Pro Tyr  
1 5

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

Ser Ser Leu Gly Gly Ser Glu Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:155:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

Ser Ser Leu Gly Gly Ser Glu Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:156:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

Ser Ser Leu Gly Gly Ser Val Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:157:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

Ser Ser Leu Gly Gly Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:158:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

Pro Ser Gln Arg His Gly Ser Lys Tyr Leu Ala Thr Ala  
1 5 10

(2) INFORMATION FOR SEQ ID NO:159:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Ser Gly Asp Arg Gly Ala  
1 5 10 15

Pro

(2) INFORMATION FOR SEQ ID NO:160:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

Leu	Asp	Ser	Ile	Gly	Arg	Phe	Phe	Ser	Gly	Asp	Arg	Gly	Ala	Pro
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:161:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

Asp	Thr	Pro	Tyr	Leu	Asp	Ile	Thr	Tyr	His	Phe	Val	Met	Gln	Arg	Leu
1				5					10						15

Pro Leu

(2) INFORMATION FOR SEQ ID NO:162:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..11
- (D) OTHER INFORMATION: /note= "where Xaa=AcNH"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

Xaa	Ala	Ser	Gln	Lys	Arg	Pro	Ser	Gln	Arg	His	Gly	Ser	Lys	Tyr	Leu
1				5					10						15

Ala	Thr	Ala	Ser	Thr
				20

(2) INFORMATION FOR SEQ ID NO:163:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 13 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide



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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:164:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

Ala His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:165:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

Val Ala Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:166:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

Val His Ala Phe Lys Asn Ile Val Thr Pro Arg Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:167:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

Val His Phe Ala Lys Asn Ile Val Thr Pro Arg Thr Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:168:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

Val His Phe Phe Ala Asn Ile Val Thr Pro Arg Thr Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:169:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:

Val His Phe Phe Lys Ala Ile Val Thr Pro Arg Thr Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:170:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

Val His Phe Phe Lys Asn Ala Val Thr Pro Arg Thr Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:171:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

Val His Phe Phe Lys Asn Ile Ala Thr Pro Arg Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:172:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

Val His Phe Phe Lys Asn Ile Val Ala Pro Arg Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:173:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

Val His Phe Phe Lys Asn Ile Val Thr Ala Arg Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:174:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

Val His Phe Phe Lys Asn Ile Val Thr Pro Ala Thr Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:175:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Ala Pro  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:176:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Ala  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:177:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:

Leu Arg Gly Ala Asn  
1 5

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WHAT IS CLAIMED IS:

1. A method for treating a host suffering from a demyelinating autoimmune disease, said disease resulting from the destruction of native tissue by a restricted  
5 population of T-cells, said restricted population comprising not less than 50% of the T-cell population, expressing no more than 12  $V_\alpha$  or  $V_\beta$  region genes, said method comprising:  
administering to said host an effective amount of a proteinaceous agent capable of reducing the number of  
10 complexes formed between a shared T-cell receptor variable region of said restricted population of T-cells and the antigen recognized by said shared T-cell receptor variable region;  
wherein said number of complexes and said destruction of  
15 native tissue is reduced.
2. A method according to Claim 1, wherein said agent is an oligopeptide comprising not more than about 30 amino acids of the amino acid sequence of said shared T cell receptor variable region.
- 20 3. A method according to Claim 2, wherein said demyelinating autoimmune disease is multiple sclerosis; and said amino acid sequence comprises the CDR3 region of a T-cell receptor  $V_\beta$  region joined to a protein other than a T-cell receptor b-subunit sequence.
- 25 4. A method according to Claim 3, wherein said amino acid sequence comprises LCASSLRGA, LCASSLRLA, or LCASSLGG.
5. A method according to Claim 1, wherein said agent is an oligopeptide comprising an amino acid sequence of mammalian myelin basic protein residues 89-99;  
30 said sequence being modified by substituting alanine for an amino acid, resulting in a sequence which, relative to said native sequence, has a substantially reduced MHC

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binding affinity, a reduced ability to stimulate T-cells in vitro, and a reduced ability to induce autoimmune disease.

6. A method according to Claim 5, wherein said oligopeptide comprises the amino acid sequence  
5 VHFAKNIVTPRTP, VHFFANIVTPRTP, VHFFKAIVTPRTP, VHFFKNIATPRTP, VHFFKNIVAPRTP, VHFFKNIVTARTP.

7. An immunogen comprising an amino acid sequence of not more than about 30 amino acids of the CDR3 region of a T-cell receptor V<sub>β</sub> region joined to a protein other than a  
10 T-cell receptor b-subunit sequence, wherein said amino acid sequence comprises LCASSLRGA, LCASSLRLA, or LCASSLGG.

8. An oligopeptide immunogen of from 9-15 amino acids comprising the amino acid sequence VHFAKNIVTPRTP, VHFFANIVTPRTP, VHFFKAIVTPRTP, VHFFKNIATPRTP, VHFFKNIVAPRTP,  
15 VHFFKNIVTARTP.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/10728

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6 C07K14/725 C07K14/47 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>THE JOURNAL OF IMMUNOLOGY,  vol.148, no.5, 1 March 1992, BALTIMORE MD,  USA  pages 1359 - 1366  R. MARTIN ET AL. 'Diversity in fine  specificity and T cell receptor usage of  the human CD4+ cytotoxic T cell response  specific for the immunodominant myelin  basic protein peptide 87-106.'  see abstract  see figures 3,4  see table II</p> <p style="text-align: center;">--- -/--</p>	1,5,6,8

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*A\* document member of the same patent family

Date of the actual completion of the international search

16 February 1995

Date of mailing of the international search report

23. 02. 95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Nooij, F

## INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/US 94/10728

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY, vol.62, no.1, January 1992, NEW YORK NY, USA pages 1 - 7 S. BROSTOFF ET AL. 'T cell receptors, immunoregulation, and autoimmunity.' see the whole document ---	1-4,7
Y	WO,A,93 12814 (THE IMMUNE RESPONSE CORP.) 8 July 1993 see claims 1,2,10-13 ---	1-4,7
A	THE JOURNAL OF EXPERIMENTAL MEDICINE, vol.173, January 1991, NEW YORK NY, USA pages 19 - 24 R. MARTIN ET AL. 'A myelin basic protein peptide is recognized by cytotoxic T cells in the context of four HLA-DR types associated with multiple sclerosis.' see abstract see figures 1,4 ---	1-8
A	NEUROLOGY, vol.43, no.4 S2, April 1993, MINNEAPOLIS MN, USA pages A411 - A412 T. YAMAMURA ET AL. 'T-cell receptor CDR3 homology among T lymphocytes capable of inducing autoimmune encephalomyelitis.' see abstract 975S ---	1-8
A	WO,A,92 21367 (A. VANDENBARK) 10 December 1992 see table 16 see claims -----	1-8



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/ 10728

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1-6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No

PCT/US 94/10728

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9312814	08-07-93	AU-B- 3418893	28-07-93
		CA-A- 2126686	08-07-93
		EP-A- 0623025	09-11-94
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WO-A-9221367	10-12-92	AU-A- 2147292	08-01-93
		CA-A- 2110055	10-12-92
		EP-A- 0587735	23-03-94
		NO-A- 934320	27-01-94
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